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AGENDA 2020: NITROGEN USE EFFICIENCY FOR LONG TERM SUSTAINABILITY OF SHORT ROTATION HYBRID POPLAR PLANTINGS

Agenda 2020

Nitrogen Use Efficiency for Long-term Sustainability of Short Rotation Hybrid Poplar Plantings

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Nitrogen Use Efficiency for Long-term Sustainability of Short Rotation Hybrid Poplar Plantings

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The rapid growth of the hybrid poplar is partially due to their genetics and partially due to the intensive cultural practices employed. Poplar plantations have typically been established on converted agricultural lands consisting of high fertility and abundant groundwater. Productivity on these sites has approached 10 green tons/acre/year with an average of 90 green tons/acre at a harvest age of 8 years. Dimensionally, the trees are over 70 feet tall and have stem diameters of 10 inches. In order to maintain these high productivity rates, we need to: 1) better understand the nutritional requirements of the poplars, especially nitrogen; and 2) identify existing hybrids that are more efficient in their nutrient uptake and use. Additional justification for studying poplar nutrition is the increasing use of hybrid poplars in environmental plantings such as: 1) riparian buffers to absorb nutrients and other chemicals coming from agricultural areas; 2) secondary treatment of sewage wastewater, and biosolids disposal; and 3) carbon sequestration. For 1) and 2), hybrid clones with high nutrient requirements as well as tolerance to salinity and other chemicals would be needed. The proposed research will provide information that can be applied to other tree species that don't possess these unique characteristics. In addition, the proposed research will provide critical physiological information that can be linked to the Department of Energy's sequencing of the poplar genome that will begin this summer.

Research Objectives and Activities

1. Determine nitrogen requirements and nitrogen use efficiency (NUE) of selected commercial hybrid poplar clones.
2. Using clones with extreme NUE, determine physiological and genetic controls of nitrogen uptake and use.
3. Develop a rapid method for determining nitrogen levels in the field.

Objective 1: Determine nitrogen requirements and nitrogen use efficiency (NUE) of selected commercial hybrid poplar clones.

Study Design

The field study was established in spring of 2003 with six commercial hybrid poplar clones representing a range of parental genetics: *Populus trichocarpa* x *P. deltoides* (TD: 57-276, PC-2 and 184-411), *P. deltoides* x *P. nigra* (DN: OP-367), *P. deltoides* x *P. trichocarpa* (DT: GR-4284), *P. trichocarpa* x *P. nigra* (TN: 311-93). The planting was a replicated nested block design: each block, replicated twice, consists of 5 nitrogen fertilizer plots (Fig. 1). Nitrogen levels based on the production levels now in use by Potlatch (i.e. 1x) are 0.3x, 0.6x, 1x, 2x, and 3x (Tab. 1). The 1x treatment was 30, 50 and 70 lbs N/a for years 1, 2 and 3, respectively. Within each fertilizer plot, 6 clonal plots were randomly established consisting of 49 trees (7 rows by 7 trees per row; at 7' x

10'spacing for 184-411, GR-4284 and 311-93, and at 14' x 10' spacing for 57-276, PC-02 and OP-367).

	Block 1 - eastside					Block 2 - west side				
south	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5
N TRT =	1X	0.6X	3X	2X	0.3X	3X	2X	0.3X	0.6X	1X
	PC-2	OP-367	184-411	PC-2	57-276	57-276	184-411	57-276	PC-2	OP-367
	184-411	184-411	GR-4284	GR-4284	OP-367	311-93	PC-2	GR-4284	184-411	184-411
	OP-367	57-276	311-93	OP-367	311-93	GR-4284	GR-4284	311-93	57-276	PC-2
	57-276	GR-4284	57-276	311-93	184-411	PC-2	311-93	184-411	OP-367	GR-4284
	GR-4284	311-93	PC-2	57-276	PC-2	OP-367	57-276	OP-367	311-93	57-276
north	311-93	PC-2	OP-367	184-411	GR-4284	184-411	OP-367	PC-2	GR-4284	311-93

Figure 1. Plot lay out showing nitrogen fertilizer plots and location of hybrid poplar clones.

Table 1. Annual nitrogen fertilizer application rates based on the commercial rate (1X).

	Fertilizer Application Rate (lbs. N/acre/year)				
Year	0.3X	0.6X	1X	2X	3X
1	9	18	30	60	90
2	15	30	50	100	150
3	21	42	70	140	210

Measurements

The inner 25 trees (5 rows by 5 trees per row) constituted permanent measurement plots. Additional trees were planted at intermediate emitters along the drip lines to provide trees for destructive sampling at the end of each of the first three years. Leaf nitrogen was sampled from 5 trees per plot in August of every year, tree growth (height and diameter) of the permanent plot trees was measured annually in the winter, and whole tree harvests were conducted at the end of each growing season on two trees per plot to characterize biomass partitioning in response to N fertilization and to determine NUE (defined as biomass per unit of N).

The data were subjected to analysis of variance using a PC-based SAS statistical program.

Results

The statistical analysis conducted on the data showed significant main effects (nitrogen treatment and clone) at a $P<0.05$ for all response variables in year 1(Tab. 2), all response variables in year 2 except aboveground biomass ($P = 0.07$) and average branch biomass ($P = 0.052$) affected by nitrogen treatment and nitrogen use efficiency as affected by clone, and in year 3, all response variables except treatment affects on branch number ($P = 0.056$) and average branch biomass ($P=0.21$). Treatment by clone interactions were found to be significant in year 1 for all response variable except NUE, in years 2 and 3 only height, diameter and leaf nitrogen concentration were significant along with aboveground biomass and NUE in year 3.

Leaf nitrogen concentration across clones showed significant difference among treatments in all 3 years (Fig. 2) with the 3x treatment exhibiting the highest concentrations compared to the other treatments except in year 2 when 3x and 2x treatments had similar concentrations. The 0.3x and 0.6x treatments consistently showed the lowest concentrations except in year 1. Overall, the leaf nitrogen concentrations showed that the treatments had the effect as expected. Year 2 concentrations were higher than the other two years for unknown reasons. The trees were capable of taking up

nitrogen at the highest treatment rate (3x) as evidenced by increasing leaf concentrations up to this treatment.

Table 2. Probabilities from analyses of variance by year, treatment (TRT), clone and treatment by clone interaction (TXC) for the response variables of height (Ht), diameter (Diam), aboveground biomass (Abgrd B), branch number (Brch No), average branch biomass (Ave Brch B), leaf nitrogen concentration (Leaf N), aboveground nitrogen (Abgrd N), and nitrogen use efficiency (NUE).

Year 1	Ht	Diam	Abgrd B	Brch No	Ave Brch B	Leaf N	Abgrd N	NUE
TRT	0.048	-	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Clone	0.0001	-	0.0001	0.0001	0.0011	0.0001	0.0001	0.0001
TXC	0.0001	-	0.056	0.084	0.0114	0.0001	0.032	0.66
Year 2								
TRT	0.0001	0.0001	0.0699	0.0016	0.0522	0.0001	0.0013	0.0004
Clone	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.255
TXC	0.0001	0.0001	0.151	0.316	0.0799	0.0001	0.116	0.877
Year 3								
TRT	0.0001	0.0001	0.0232	0.056	0.207	0.0001	0.0001	0.0001
Clone	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0400	0.0034
TXC	0.0001	0.0001	0.0484	0.258	0.853	0.0001	0.228	0.0504

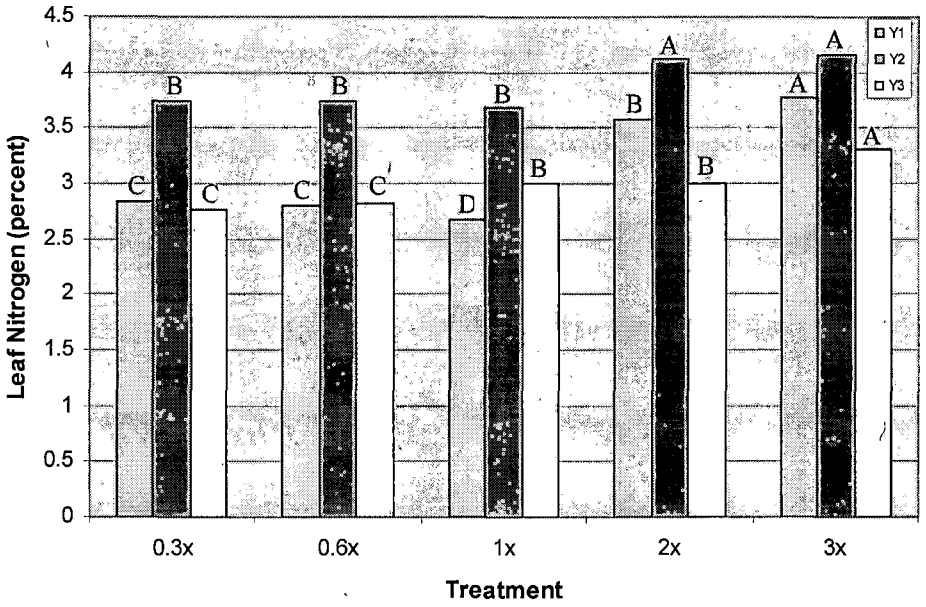

Figure 2. Leaf nitrogen concentration (%) by nominal nitrogen treatment (see Table 1) for years 1 – 3.

Clonal differences in leaf nitrogen concentration were also evident with 57-276 exhibiting overall the highest concentrations over the 3 years and GR-4284 the lowest (Fig. 3). The other clones ranked from high to low, 311-93, PC-2, OP367 and 184-411.

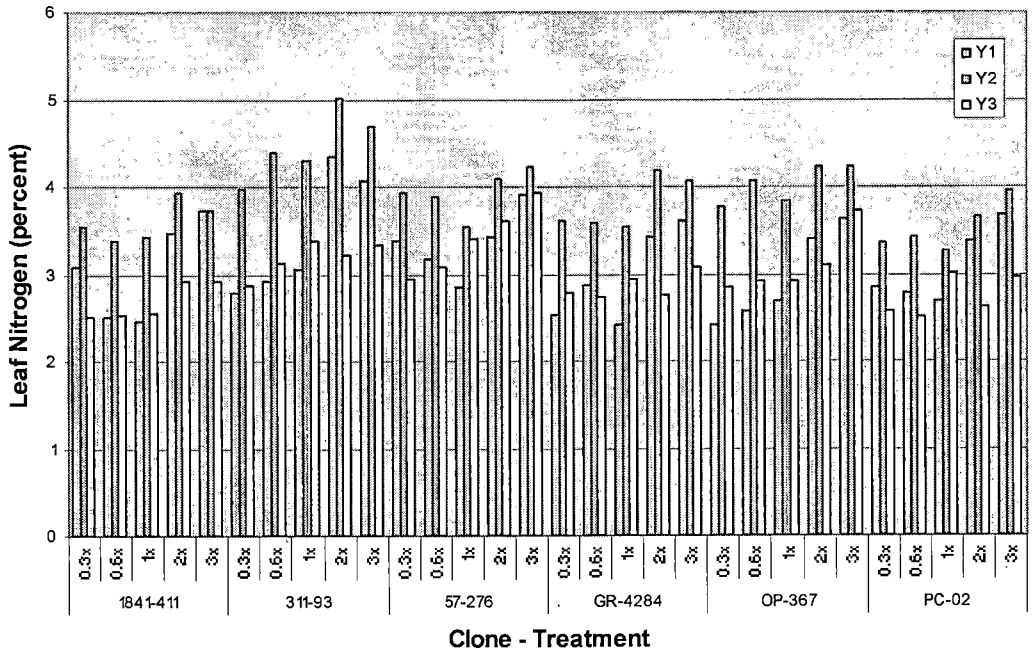

Figure 3. Leaf nitrogen concentration (%) by nominal nitrogen treatment (see Table 1) and clone for years 1 – 3.

The response in height and diameter to nitrogen treatment was variable by clone (Figs. 4 & 5). In some clones the response to nitrogen increased with increasing N, e.g. 311-93 and PC-2. However, in other clones, the 0.3x treatment showed significant height and diameter, e.g. 57-276 and OP-367. This may have been a result of soil microsite effects. In March 2007 in attempt to understand these unusual growth responses, selected plots were cored to determine the depth to water table. In plots where growth was greater than expected based on nitrogen treatment, the water table was found to be within a meter of the surface suggesting that water availability may have confounded the nitrogen and clone treatments.

Clone ranking differed by height and diameter (figs. 4 & 5). For height growth the clones ranked from tallest to shortest: 311-93, 184-411, OP-367, PC-2, GR-4284 and 57-276. Whereas with diameter growth, the rank was: OP-367, 57-276, 184-411, PC-2, 311-93 and GR-4284. Combining height and diameter into one variable, volume index, the clones ranked from highest to lowest: OP-367, 184-411, 57-276, 311-93, PC-2 and GR-4284.

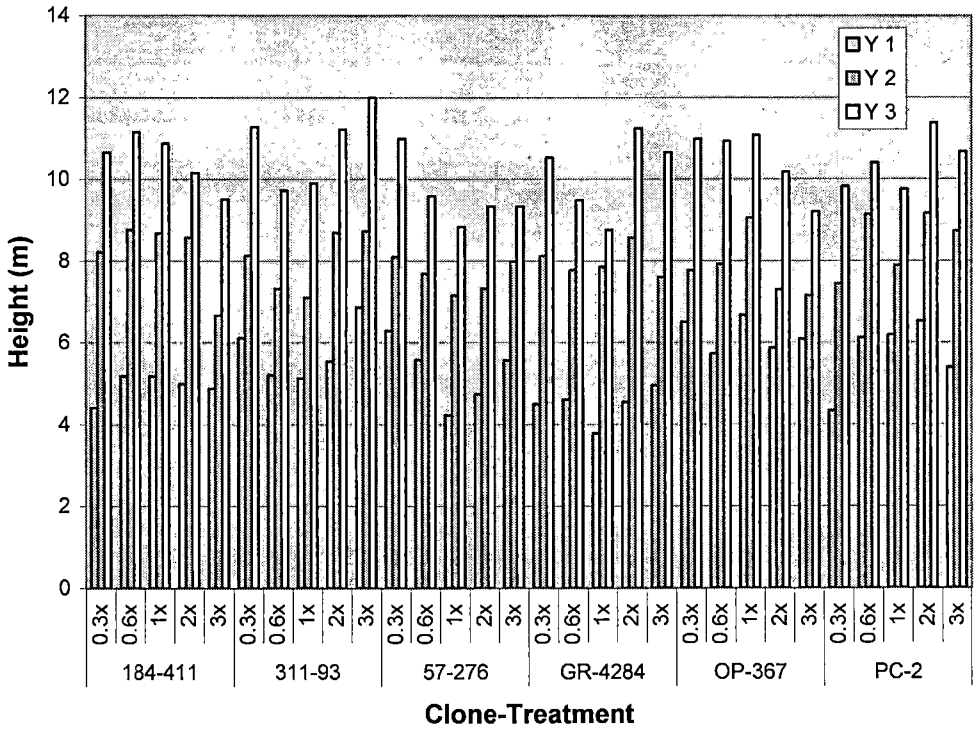

Figure 4. Dormant season height (m) by nominal nitrogen treatment (see Table 1) and clone for years 1 – 3.

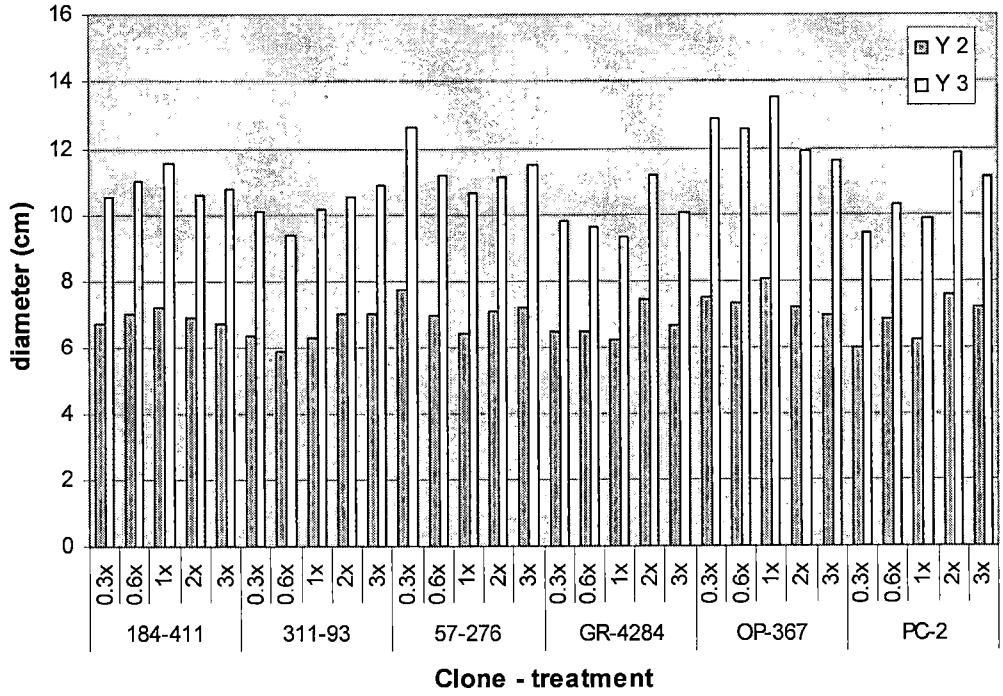

Figure 5. Dormant season diameter (cm) by nominal nitrogen treatment (see Table 1) and clone for years 2 and 3.

The aboveground biomass from the destructive harvests conducted every winter showed significant treatment and clone effects (Fig. 6). As with heights and diameters, aboveground biomass showed variable response, probably due to the confounding water effects discussed above and the fact that only 2 trees per plot (a total of 4 per treatment-clone combination) were harvested.. Some clones showed increasing biomass with increasing nitrogen, e.g. 184-411, PC-2 and 311-93 whereas the other clones showed greater biomass in the 0.3x treatment. Clone effects were similar to the volume index reported above with OP-367 exhibiting the greatest biomass followed by 57-276, PC-2, 184-411, 311-93 and GR-4284.

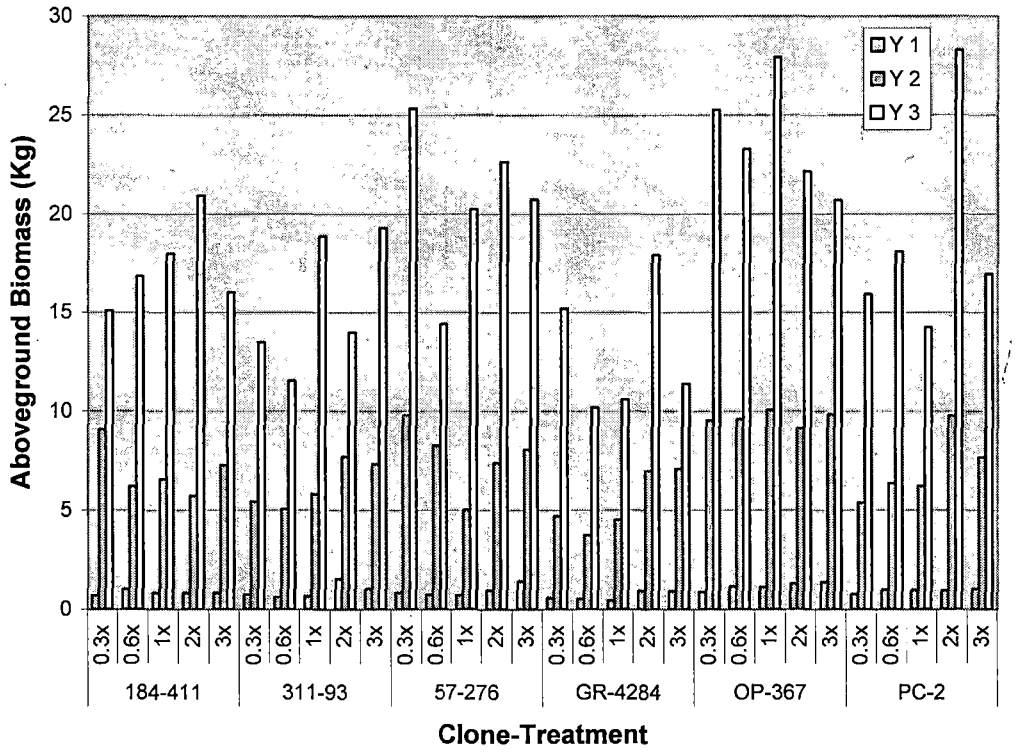

Figure 6. Aboveground biomass (Kg) by nominal nitrogen treatment (see Table 1) and clone for years 1 – 3.

Branch production has been shown to be a sensitive variable to nitrogen fertilization with higher numbers of branches being produced with increasing N levels. This was the general response found in this study (Fig. 7). Branch number is also under genetic control with a very wide range in branch number observed among the clones. For example, in year 3, the number of branches per tree ranged from 118 in 311-93 to 71 in PC-2. In general, hybrids with a P. nigra parent tend to have more branches such as 311-93, which is a hybrid between P. trichocarpa and P. nigra. In contrast, P. deltoides tend to have fewer branches as evidenced by GR-4284, a hybrid between a P. deltoides mother and a P. trichocarpa father, which had 82 branches. OP-367 is a hybrid between P. deltoides and P. nigra and the number of branches was intermediate at 105.

Average branch biomass is another response variable that is both under genetic control, but also responsive to nitrogen fertilizer (Fig. 8). Clone ranking in this variable mirrored aboveground biomass and can be explained by the crown architecture needed to

display leaves which drive growth. From highest to lowest average branch biomass by clone was: OP-367, PC-2, 57-276, 184-411, 311-93 and GR-4284.

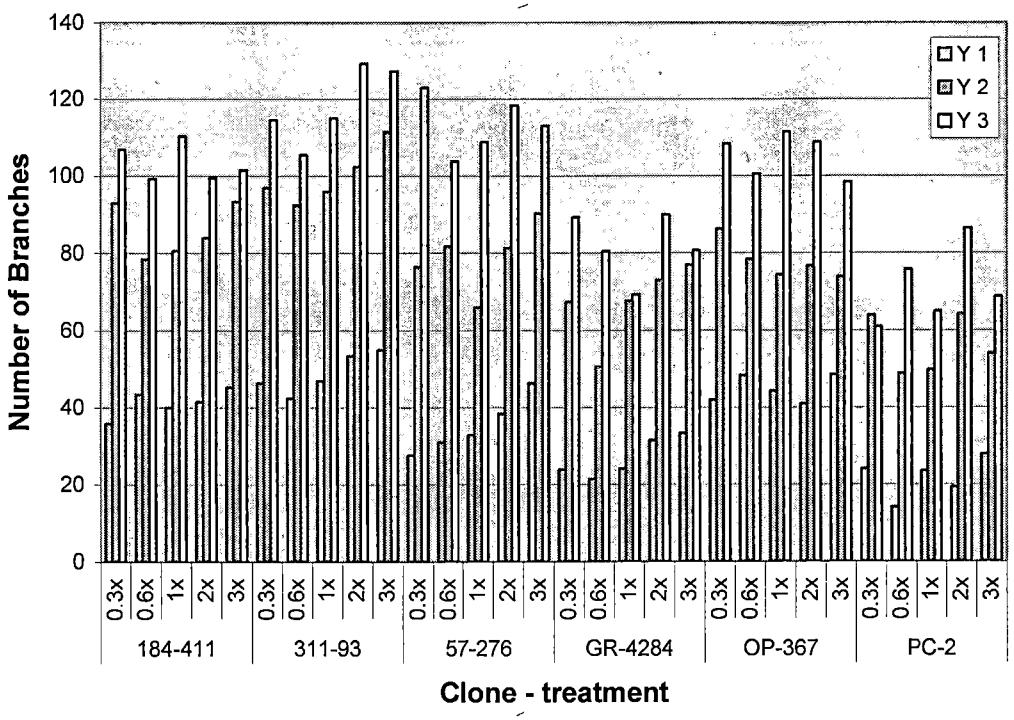

Figure 7. Number of branches per tree by nominal nitrogen treatment (see Table 1) and clone for years 1 – 3.

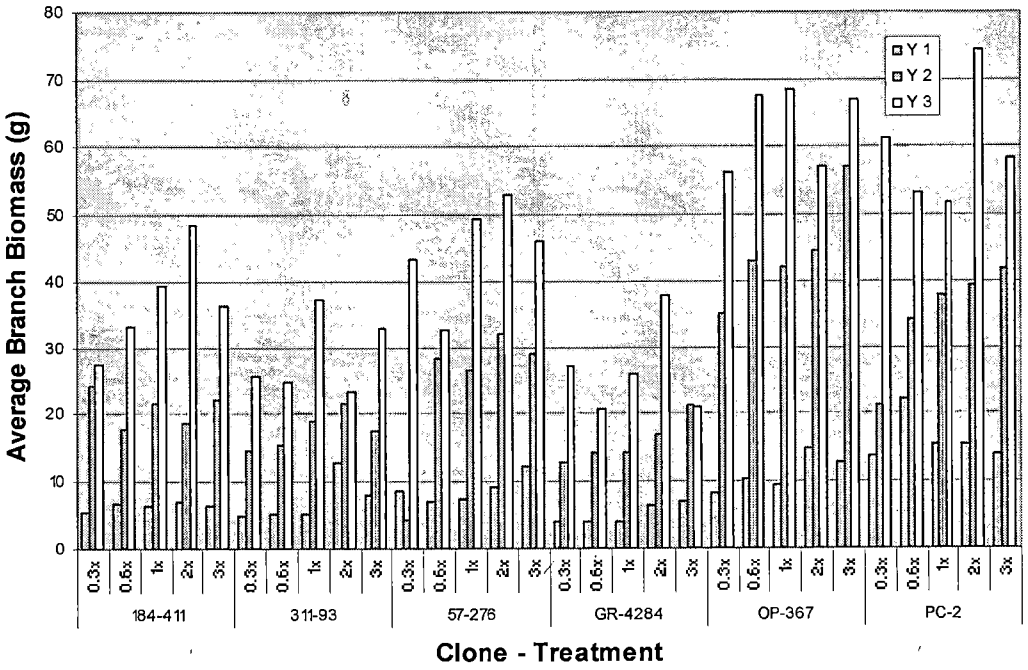

Figure 8. Average branch biomass (g) per tree by nominal nitrogen treatment (see Table 1) and clone for years 1 – 3.

Aboveground nitrogen content exhibited increasing amounts from 0.3x, but usually peaked at 1x (184-411, OP-367) or 2x (57-276, GR-4284, PC-2). The clones in year 3 ranged from PC-2 (164 g) to GR-4284 (97 g) with OP-367 (161 g), 57-276 (154 g) and 184-411 (130 g) ranking 2nd to 4th. Clone 311-93 had the next to lowest N content at 108 g).

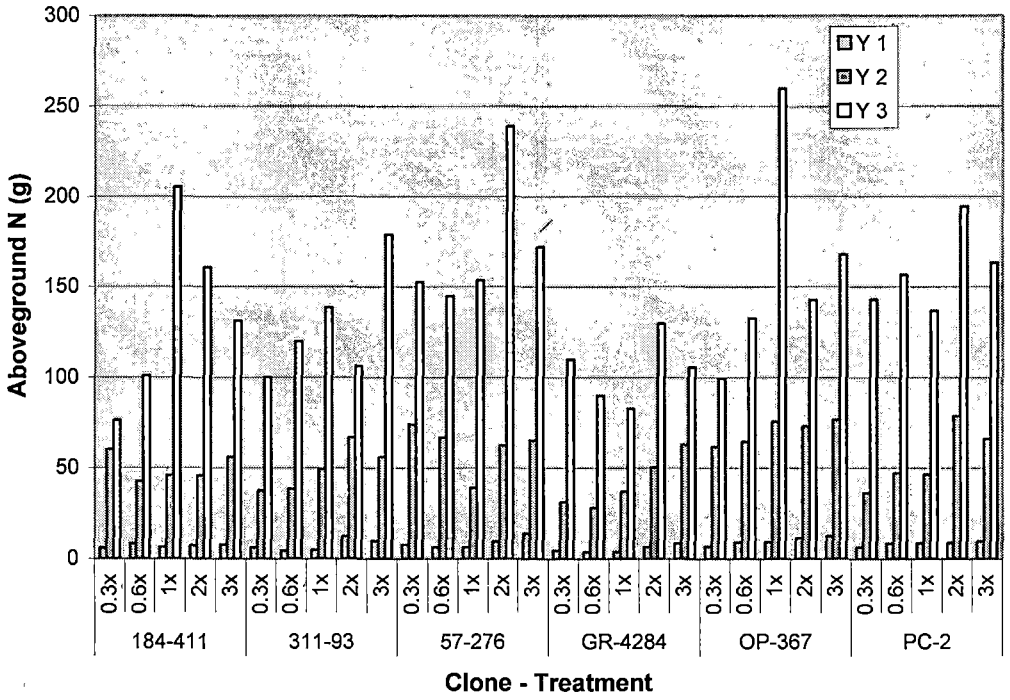

Figure 9. Aboveground nitrogen content (g) per tree by nominal nitrogen treatment (see Table 1) and clone for years 1 – 3.

In order to understand nitrogen use by the clones as affected by the nitrogen treatments, nitrogen use efficiency was used in the analysis. NUE is defined as the aboveground biomass divided by aboveground N with the units being Kg/Kg. Typically, NUE decreases as nitrogen fertilization goes up because the trees will take up excess N, but it may not be used in producing biomass (Fig. 10). NUE ranged from 140 in the 0.3x treatment down to 115 in the 3x treatment. This trend held up except for the 0.3x treatment in year 1 and 2x in year 3.

NUE was significantly affected by clone (Fig. 11). The ranking in NUE by clone was from highest to lowest: GR-4284, 311-93, OP-367, 184-411, PC-2 and 57-276. This information can be used to select clones where nitrogen may be limiting growth or where lower rates of fertilization may be used to reduce production costs. On the other hand, in situations where there may be excess nitrogen present, such as in environmental plantings and riparian buffers, clones with low NUE could be planted to ensure uptake of the excess N.

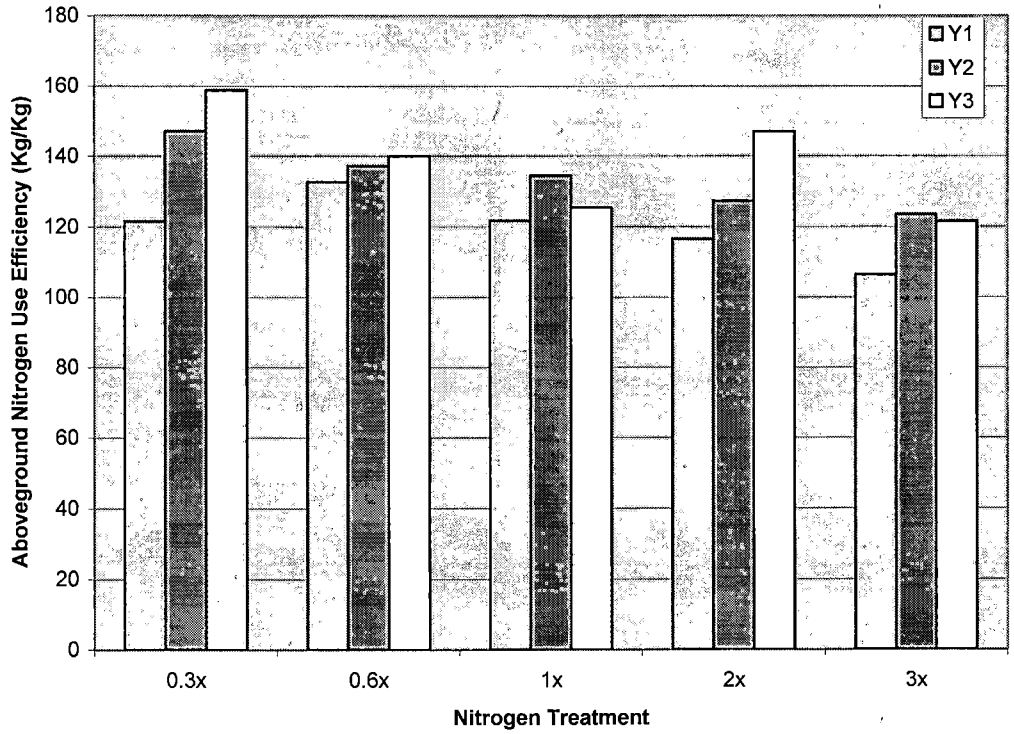

Figure 10. Aboveground nitrogen use efficiency (Kg biomass/Kg nitrogen) per tree by nominal nitrogen treatment (see Table 1).

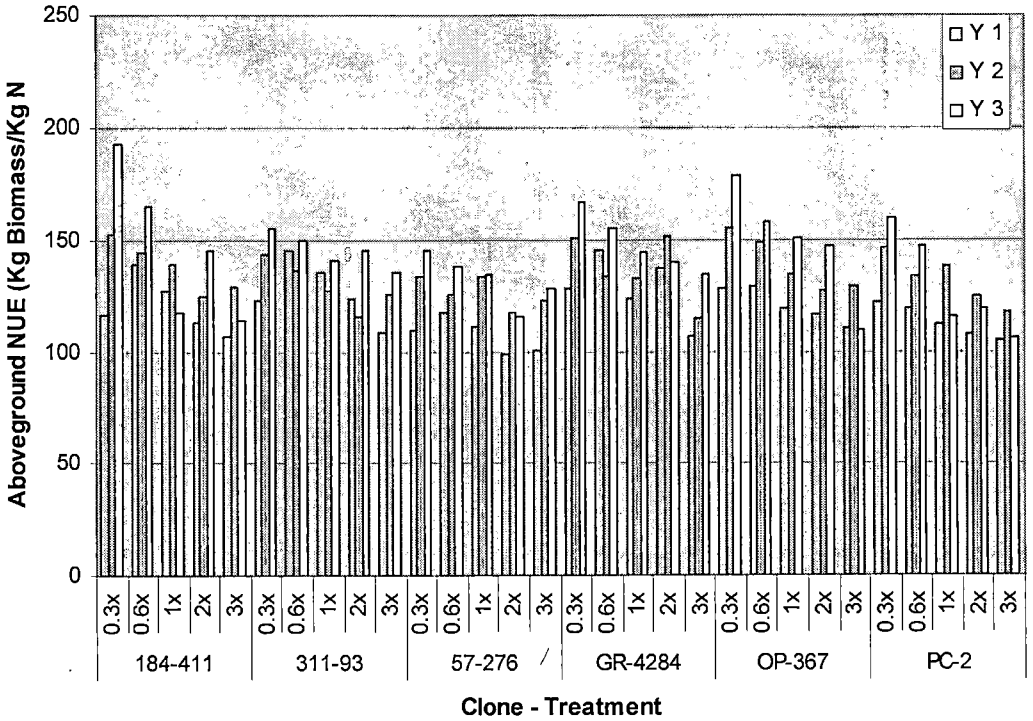

Figure 11. Aboveground nitrogen use efficiency (Kg biomass/Kg nitrogen) per tree by nominal nitrogen treatment (see Table 1) and clone for years 1 – 3.

Objective 2: Using clones with extreme NUE, determine physiological and genetic controls of nitrogen uptake and use.

This objective was addressed by a Master of Science graduate student, Shan Zhao, whose thesis has been attached (Appendix A). Below is a summary of the findings of this research.

Hybrid poplars are the result of interspecific crosses in the *Populus* genus and are genetically predisposed to grow faster and have wider adaptability than either parent species. They have been widely adopted for use in short rotation plantations in the Pacific Northwest for chips to make paper and for solid wood products. Nitrogen (N) can be one of the most limiting factors for growth and therefore precise N management is needed. In the present study, the physiological control of N use and a field-portable method of estimating N level of hybrid poplars were investigated in two greenhouse studies. Two hybrid poplar clones GR-4284 (*Populus. deltoides* × *P. trichocarpa*) and 57-276 (*P. trichocarpa* × *P. deltoides*) were selected based on a previous field study as plant materials for this study.

In this experiment, the clonal and physiological responses of 57-276 and GR-4284 to N were examined. The steady-state experimental technique with a customized semi-hydroponic system was used to provide nutrients. Two relative addition rates (RAR) 1.5% and 10%, were used to create low and high N treatments. When external N changes from low to high, there were clonal differences in the mechanism of the growth response. 57-276 depended more on increasing the productivity per unit of N, and GR-4284 increased the amount of N present in the plant, especially in leaves. The productivity per unit N in 57-276 increased by increasing leaf N productivity. Neither the leaf N nor the N productivity of GR-4284 responded to the N addition. There was no clonal difference in the response of photosynthetic N use efficiency to N addition rate.

In comparison with GR-4284, clone 57-276 was more sensitive to low N, but became more responsive when N increased. This response was attributed to different growth mechanisms exhibited when the N level was increased. Clone 57-276 increased productivity per unit leaf area and the productivity per unit N, while clone GR-4284 relied more on leaf area expansion and increased plant N to increase growth.

Objective 3: Develop a rapid method for determining nitrogen levels in the field.

Background

Next to water, nitrogen is the most limiting site factor for the growth of plants, including hybrid poplar. Leaf nitrogen concentrations of less than 2.75% have been suggested for hybrid poplar as being deficient and at which tree growth begins to suffer. Currently, the most accurate method for determining nitrogen is both cost prohibitive and time consuming when managing and measuring large acreages of poplar. In this study, we used the Minolta SPAD-502 Leaf Chlorophyll meter that determines the relative amount of chlorophyll and compared it to a laboratory based analytical method. The advantage of the SPAD meter is that it's rapid, requiring only seconds to measure a leaf, it is non-destructive, and inexpensive. Good correlations between the meter reading and

leaf N concentration have been reported in annual crops including wheat, corn, rice and cotton.

Measurements

Every August over the 3 year study period, one leaf was sampled from each of 5 random trees per plot. Each leaf was measured 10 times in the field with a Minolta SPAD Model 502 Chlorophyll meter and the mean chlorophyll content recorded. The leaves were immediately sealed into plastic bags and placed on ice in a cooler. The leaves were transported to the lab where two leaf disks of 2.54 cm in diameter were taken using an arch punch. The leaf disks and the remainder of the leaves were dried for at least 48 hours at 60C. The disks were weighed on an analytical balance. Specific leaf area (SLA) was determined by dividing the area of the two leaf disks by their cumulative dry weight. The dried leaves were ground in a Wiley Mill using a 20 mesh screen. The ground leaf tissue was then analyzed for total nitrogen on a CE Carbon-Nitrogen Analyzer, Model 2100 Soil. The C-N analyzer provides nitrogen concentration based on the weight of sample used. This was converted to an area based nitrogen concentration by dividing the weight-based N concentration by the SLA.

The data were subjected to analysis of variance followed by regression analysis using a PC-based SAS statistical program.

Results

The relationships between SPAD values and weight-based leaf nitrogen concentrations provided significant linear equations (Fig. 12), but the relationships were clone-specific. The coefficients of determination (R^2) for the clone equations ranged from 0.77 for GR-4284 to 0.41 for PC-2 (Tab. 3). The slopes ranged from 0.115 for GR-4284 to 0.075 for PC-2 (Fig. 12).

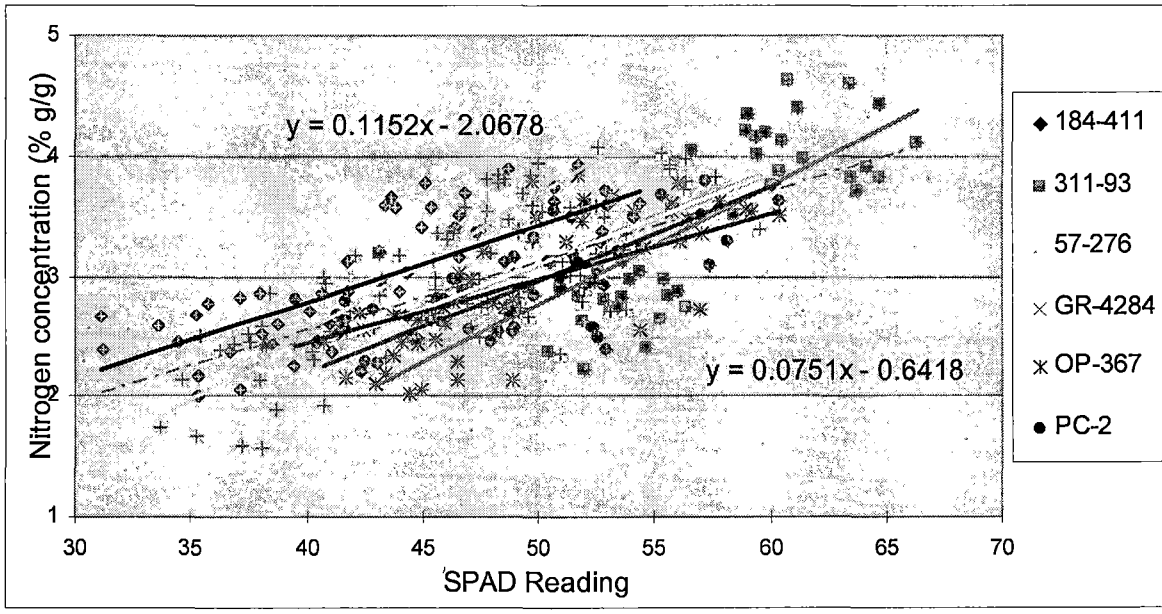

Figure 12. The relationship between SPAD meter readings (unitless) and leaf nitrogen concentrations (weight-based percent) for 6 hybrid poplar clones grown at 5 nitrogen fertilization rates (see Table 1). The two regression equations represent the extremes: upper equation = GR-4284 and lower equation = PC-2.

Specific leaf area provides the conversion between leaf area and leaf weight and shows significant clone effects, but little to no nitrogen treatment effects (Fig. 13). Once again GR-4284 and PC-2 are at the extremes with SLAs of 137 cm^2/g and 118 cm^2/g, respectively. The higher the SLA, the thinner the leaf, i.e. more leaf area for unit leaf weight, and the lower the SLA the thicker the leaf.

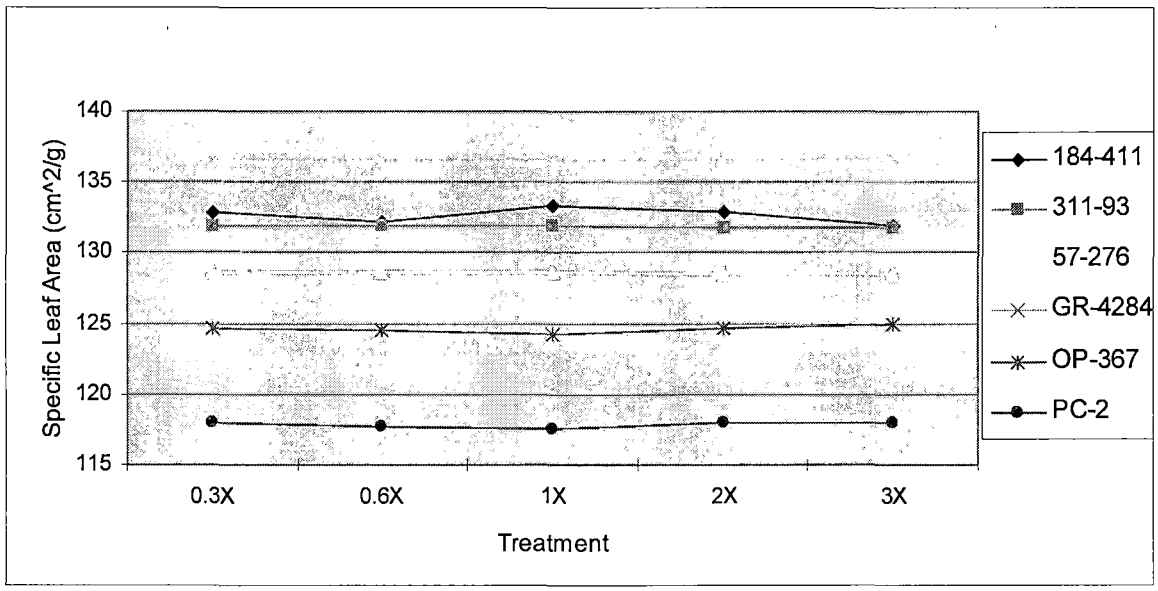

Figure 13. Specific leaf area (cm^2/g) as affected by nitrogen treatment and clone.

Using SLA to convert weight-based leaf nitrogen concentrations to area-based concentrations and replotting the SPAD data provided a much better relationship with less clonal effect (Fig. 14). The R^2 values improved in 4 of the 6 clones and in the regression equation that combined all of the clones (Tab. 3). Only clone 57-276 showed a poor relationship based on an R^2 of 0.40.

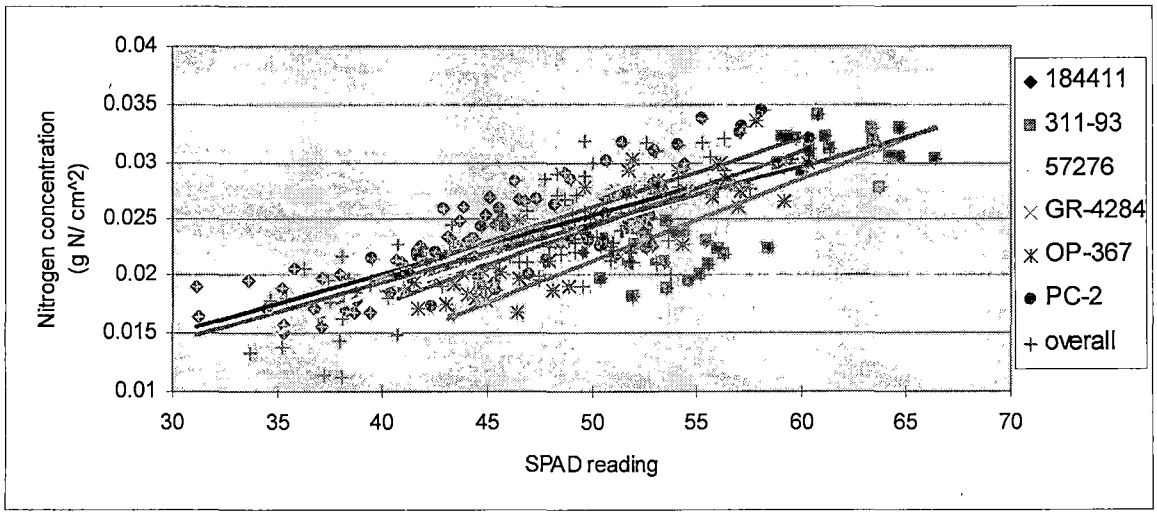

Figure 14. The relationship between SPAD meter readings (unitless) and leaf nitrogen concentrations (leaf area-based percent) for 6 hybrid poplar clones grown at 5 nitrogen fertilization rates (see Table 1).

Table 3. Coefficient of determination (R^2) for linear regressions between SPAD meter values and leaf nitrogen based on either weight or area.

R^2	184-411	311-93	57-276	GR4284	OP367	PC-2	Combined
leaf weight based	0.55	0.59	0.42	0.77	0.58	0.41	0.48
Leaf area based	0.66	0.65	0.40	0.70	0.71	0.57	0.60
percent change	20.62	9.46	-5.23	-8.21	22.72	38.44	25.00

Conclusions

The Minolta SPAD-502 chlorophyll meter can be used to estimate hybrid poplar foliage N concentrations using linear regression equations. However, specific leaf area needs to be factored into the SPAD-N model, especially for thick leaf clones, like 57-276. At similar leaf N concentrations, SPAD readings increased with leaf thickness, as a result of measurement principles of the SPAD-502 meter. Using area-based leaf N concentration to establish the SPAD-N relationships can improve the accuracy of the regression models.

Appendix A

NITROGEN NUTRITION OF HYBRID POPLARS

By

SHAN ZHAO

A thesis submitted in partial fulfillment of
the requirements for the degree of

Master of Science in Natural Resource Sciences

WASHINGTON STATE UNIVERSITY
Department of Natural Resource Sciences

August 2006

To the faculty of Washington State University:

The members of the Committee appointed to examine the thesis of SHAN ZHAO find it satisfactory and recommend that it be accepted.

_____

Chair

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ACKNOWLEDGEMENT

I would like to thank Dr. Jon Johnson for persevering with me throughout the time it took me to do the experiments and write my thesis. He has generally given his time and expertise to better my work.

I'm also grateful to my academic committee members: Dr. John Bassman, Dr. Gerald Edwards and Dr. Shiou Kuo. I thank them for their advices and support.

Last but not the least; I thank Rod Clausen, the program coordinator, for his overseeing the administrative concerns, so that it's possible for me to finish my degree 300 miles away from Pullman.

NITROGEN NUTRITION OF HYBRID POPLARS

Abstract

By Shan Zhao, M.S.
Washington State University
August 2006

Chair: Jon D. Johnson

Hybrid poplars are the result of interspecific crosses in the *Populus* genus and are genetically predisposed to grow faster and have wider adaptability than either parent species. They have been widely adopted for use in short rotation plantations in the Pacific Northwest for chips to make paper and for solid wood products. Nitrogen (N) can be one of the most limiting factors for growth and therefore precise N management is needed. In the present study, the physiological control of N use and a field-portable method of estimating N level of hybrid poplars were investigated in two greenhouse studies. Two hybrid poplar clones GR-4284 (*Populus. deltoides* × *P. trichocarpa*) and 57-276 (*P. trichocarpa* × *P. deltoides*) were selected based on a previous field study as plant materials in two experiments.

In experiment 1, the clonal and physiological responses of 57-276 and GR-4284 to N were examined. The steady-state experimental technique with a customized semi-hydroponic system was used to provide nutrients. Two relative addition rates (RAR) 1.5% and 10%, were used to create low and high N treatments. Results showed clonal response to N. In comparison with GR-4284, clone 57-276 was more sensitive to low N, but more responsive when N increased. This response can be attributed to different growth mechanisms exhibited when the N level was increased. Clone 57-276 increased productivity per unit leaf area and the productivity per unit N, while clone GR-4284 relied more on leaf area expansion and increased plant N to increase growth.

Experiment 2 examined the use of Minolta SPAD-502 chlorophyll meter to estimate hybrid poplar leaf N level. RAR of 1.5% and 15% were used to create low and high N treatments. Experimental results showed that the SPAD-502 chlorophyll meter can be used to estimate the hybrid poplar leaf N level using a significant linear relationship between SPAD readings and N concentration. SPAD readings were found to be affected by leaf thickness at similar N concentration, where SPAD readings decreased as specific leaf

area increased. Therefore, specific leaf area needs to be considered into the SPAD-N model, especially for thick leaf clones.

TABLE OF CONTENTS

ABSTRACT vi

TABLE OF CONTENTS vii

LIST OF TABLES viiii

LIST OF FIGURES ix

CHAPTERS

1 INTRODUCTION 2

2 LITERATURE REVIEW 4

2.1 Review of researches on plant N utilization 4

2.2 Review of steady state experimental technique 5

2.3 Review of SPAD-502 chlorophyll meter application 8

3 RESEARCH DESIGN AND METHODOLOGY 10

3.1 Research objectives 10

3.2 Research design and methodology 12

3.2.1 Experiment 1 research materials and methods 13

3.2.2 Experiment 2 research materials and methods 15

3.3 Statistical analysis 16

4 RESULTS AND DISCUSSION 17

4.1 Experiment 1 results and discussion 17

4.1.1 Steady-state test 17

4.1.2 Plant growth response to N treatments 19

4.1.3 N productivity and plant N concentration 26

4.2 Experiment 2 results and discussion 32

4.3 Conclusions and Recommendations 41

BIBLIOGRAPHY 42

APPENDIX

Appendix □ List of parameters used with their symbols, units and definitions 38

Appendix □ The p-value of parameter changes from 1.5% to 10% RAR 38

LIST OF TABLES

Table 1. Relative addition rate (RAR gN g N^{-1} day^{-1}), relative growth rate (RGR, g g^{-1} day^{-1}), relative uptake rate (RUR g g N^{-1} day^{-1}) and plant N concentration (PNC, mg g^{-1}) of clone 57-276 and GR-4284 14

Table 2. The regression coefficients of shoot-root relationship for clones 57-276 and GR-4284 at 1.5% and 10% RAR. The model was: Ln(shoot)=slope×Ln(root) + intercept 15

Table 3. Plant dry weight (g) of clones 57-276 and GR-4284 at each harvest 16

Table 4. Probability of biomass differences between clones 57-276 and GR-4284 at each harvest 16

Table 5. Linear correlation between SPAD and acetone-extracted chlorophyll concentration for clone 57-276, GR-4284 and their combination 28

Table 6. Linear regression model between foliar N concentration and acetone-extracted chlorophyll concentration for two hybrid poplar clones, 57-276 and GR-4284 29

Table 7. Linear regression model between SPAD values and foliar N concentration for two hybrid poplar clones,57-276 and GR-4284 30

Table 8. Linear correlation between SPAD values and specific leaf area for selected leaves with similar N concentration of clone 57-276 at two RAR levels 31

Table 9. The parameters and their p-values in SPAD-N model for two hybrid poplar clones, 57-276 and GR-4284 32

Table 10. Linear regression equations among SPAD values, area based N concentration, and area based chlorophyll concentration, for clone 57-276 33

Table 11. Linear regression equations among SPAD values, area based N concentration, and area based chlorophyll concentration for clone GR-4284 33

LIST OF FIGURES

Figure 1. The customized semi-hydroponic system used in both experiments 11

Figure 2. The amount of N added (mg) in (a) 1.5% RAR treatment (b) 10% RAR treatment over a 9 week period 12

Figure 3. Plant N concentration (PNC, mg g^{-1}) of clone 57-276 and GR-4284 at each harvest date 15

Figure 4. The allometric relationships between the shoot and root dry weight of clones 57-276 and GR-4284 grown at (a)1.5% RAR and (b) 10% RAR 15

Figure 5. Total plant dry weight (ln dw) accumulation over time for clones 57-276 and GR-4284 grown at two different relative addition rates (RAR). The ln(dw) drop at 2^{nd} and 4^{th} harvest of 1.5% treatments was due to variability in plant size 16

Figure 6. Relative growth rate (RGR, g g^{-1} day^{-1}) of clones 57-276 and GR-4284 at 1.5% and 10% relative addition rates. 17

Figure 7. Net assimilation rate (NAR, g cm^{-2} day^{-1}) of clones 57-276 and GR-4284 at different plant N concentration 18

Figure 8. Leaf weight ratio (LWR) of clones 57-276 and GR-4284 at low and high leaf N concentration 18

Figure 9. Specific leaf area (SLA, cm^2 g^{-1}) of clones 57-276 and GR-4284 at low and high leaf N concentration 19

Figure 10. The GRC_{LWR}, GRC_{SLA}, GRC_{LAR} and GRC_{NAR} of clones 57-276 and GR-4284 20

Figure 11. Photosynthetic rate (A μmol CO_2 m^{-2} s^{-1}) of 57-276 and GR-4284 at two levels of leaf N concentration (gN m^{-2}) 21

Figure 12. N productivity (Pn, g plant gN^{-1} day^{-1}) of clones 57-276 and GR-4284 at two plant N concentrations 22

Figure 13. Plant N concentration (PNC mg g^{-1}) of clones 57-276 and GR-4284 at two relative addition rates (RAR % gN^{-1} day^{-1}) 23

Figure 14. Relative growth rate (RGR, % g^{-1} day^{-1}), plant N concentration (PNC, mg g^{-1}) and N productivity (Pn g gN^{-1} day^{-1}) of clones (a) 57-276 at 1.5% and 10% RAR and (b) GR-4284 at 1.5% and 10% RAR 24

Figure 15. Growth Response Coefficient of N productivity (Pn g gN^{-1} day^{-1}) and plant N concentration (PNC mg g^{-1}) for clones 57-276 and GR-4284 24

Figure 16. Leaf N productivity (Pnl, g g leaf N^{-1} day^{-1}) of clones 57-276 and GR-4284 at two plant N concentrations 25

Figure 17. Leaf N ratio (LNR) of clones 57-276 and GR-4284 at two plant N concentrations 25

Figure 18. Photosynthetic N use efficiency (NUE_{PH}, μmol CO_2 gN^{-1} s^{-1}) of 57-276 and GR-4284 at two leaf N concentrations (LNC, g m^{-2}) 26

Figure 19. Effects of two N relative addition rate on leaf N concentration (mg g^{-1}), SPAD values and chlorophyll concentration (mg g^{-1}) of two hybrid poplar clones, GR-4284 and 57-276 The means and standard errors of 18 samples for clone 57-276 and 9 samples for clone GR-4284 are shown 27

Figure 20. The relationship between SPAD and acetone-extracted chlorophyll concentration for two hybrid poplar clones, 57-276 and GR-4284 28

Figure 21.The relationship between foliar N concentration (mg g^{-1}) and acetone-extracted chlorophyll concentration (mg g^{-1}) for two hybrid poplar clones, 57-276 and GR-4284. 29

Figure 22. Ratio of chlorophyll to N (chl/N) as influenced by clone and relative addition rate..... 29

Figure 23. The relationship between SPAD value and foliar N concentration (mg g^{-1}) for two hybrid poplar clones, 57-276 and GR-4284..... 30

Figure 24. The relationship between SPAD readings and specific leaf area (SLA, cm^2 g^{-1}) of selected leaves with similar N concentration in clone 57-276 at (a)1.5% RAR and (b) 15% RAR..... 31

Figure 25. Effects of two N relative addition rate on the specific leaf area (cm^2 g^{-1}) of two hybrid poplar clones, GR-4284 and 57-276..... 32

Dedication

This thesis is dedicated to my parents who have taught me the attitude to life.

CHAPTER ONE

INTRODUCTION

Trees in the *Populus* genus grow aggressively and reproduce readily from seeds and cuttings on ideal sites. *P. deltoides*, known as eastern cottonwood, typically live in riparian zones. It ranges from Quebec and New England to Florida, and west to the base of the Rocky Mountains (WDNR, 2004). The name cottonwood came from the hair-like structures attached with seeds produced by female aments to facilitate seed dispersal over large distances. *P. trichocarpa* Torr. & Gray, is commonly known as black cottonwood or western balsam poplar. It is the largest American poplar and the largest hardwood tree in western North America. It ranges from Alaska, through coastal regions of western Canada into the northwestern US. Along the Pacific Coast, black cottonwood often forms extensive stands on bottomlands of major streams and rivers at low elevations.

Interspecific hybridizations in the Pacific Northwest began in 1970 with the crosses between *P. trichocarpa* and *P. deltoides* (Stettler, 1996) and has advanced during the past 20 years in the Pacific Northwest with over 50000 acres currently in commercial production (Stanton, 2002). Envisioned as a potential source for renewable energy, hybrid poplars have been grown intensively in short-rotation plantations for chips to make paper and for solid and engineered wood products. Their utility also extends to wastewater treatment, phytoremediation and carbon sequestration.

Hybrid poplars are genetically predisposed to grow faster and have wider adaptability than either parent species. On good sites, hybrid poplars grow faster than any other northern temperate region tree. They can produce 20 to 25 meter trees with 20 to 25 cm diameter in 6 to 8 years. The hybrid poplar leaves can be four times larger than leaves of either parent at the same age and on the same site. They are easily propagated from stem cuttings, but because of quick re-sprouting, replanting after harvesting may be unnecessary, especially for short harvest cycles (Nesom, 2002).

Nitrogen (N), however, can be one of the most often limiting factors for tree growth (Chang, 2002). In N deficient forests, a small increase in plant N can result in large increases in productivity, though the degree of response varies by clone. Therefore, knowing the difference in plant response to N is needed in intensive cultural practices for maximizing wood production, and reducing N contamination of ground and

surface water, and fertilizer cost. In this study, the physiological components of hybrid poplar clones grown in low and high N levels were examined, to help understand the genetic control of N use.

Detecting plant N status and requirements are critical in N management. The traditional plant tissue analysis is expensive, time consuming, and have limited utility for within-season management. Tree growers need techniques that can quickly and inexpensively diagnose N status in the field. Because much of the leaf N is associated with the photosynthesis apparatus, efforts have been made to estimate leaf N with field-portable chlorophyll meters, such as the Minolta SPAD-502 chlorophyll meter (Soil-Plant Analysis Development Section). The Minolta SPAD-502 chlorophyll meter is a field portable instrument that measures relative chlorophyll content with a value range of 0 to 100. It has been tested on some annual and perennial crops, such as corn, wheat, tomato, apple for its application for leaf N estimation. Although it cannot predict the absolute foliar N concentration, it has proven to be useful in timing of N fertilization for the some species. In the current project, the use of SPAD-502 chlorophyll meter to determine leaf N levels of hybrid poplars was studied. The relations between SPAD readings, chlorophyll content and leaf N concentration were established, and leaf properties that affect the SPAD readings were examined.

CHAPTER TWO
LITERATURE REVIEW

2.1 Review of researches on plant N utilization

Nitrogen is an important determinant of forest productivity. It plays a pivot role in many critical functions, such as photosynthesis, where it is found in chlorophyll and CO_2 – carboxylating enzymes. It is essential for protein synthesis and forming plant structure. Research has shown that growth rate and growth patterns of hybrid poplars can be affected by N availability (Bunn, 2004). Interspecific difference in N use has been explained by the variation in N uptake rate and N utilization (Miller and Hawkins, 2003).

Nitrogen use efficiency (NUE) and nitrogen productivity (P_N) are the two parameters that describe the utilization of absorbed N. NUE is the amount of dry matter produced per unit N (g gN^{-1}) and is independent of time. NUE was found to be closely related to the plant N level in three *Salix* clones (Ericsson, 1981). Variations in NUE have been related to physiological variations in photosynthetic N use efficiency, respiration rate per unit N and N allocation (Atkin, 1996). NUE was also reported to be affected by environmental factors, such as temperature and light availability (Yin, 1993).

N productivity (P_N) is the amount of biomass produced per amount of N and per unit of time (g gN^{-1} day^{-1}) (Agren, 1983). P_N considers the effect of time, and linearly relates plant relative growth rate to plant N concentration (Ingestad, 1979, Jia and Ingestad, 1984). In the N limiting range, it can be expressed as the slope of the linear relationship between relative growth rate (RGR, g g^{-1} day^{-1}) and internal N concentration. N productivity can be divided into two components: leaf N ratio (LNR), which is the proportion of the plant's N present in the leaves, and the leaf N productivity (P_{nl} g gleaf N^{-1} day^{-1}), which is defined as the increase in plant dry matter per unit time and leaf N content (Garnier and Vancaeyzeele, 1994):

$$Pn = LNR \times P_{nl} \quad (1)$$

where P_{nl} is the ratio between net assimilation rate (NAR, g cm^{-2} day^{-1}) to leaf N concentration (LNC, mg g^{-1}).

$$P_{nl} = NAR/ LNC \quad (2)$$

Because photosynthesis is a major component of NAR, P_{nl} is likely to depend on photosynthetic N use efficiency, which is the ratio of photosynthesis rate to leaf N concentration (Garnier and Vancaeyzeele, 1994).

The variation of N productivity has been reported to be affected by species (Jia and Ingestad, 1984), photon flux density (McDonald, 1992), N allocation and photosynthesis N use efficiency (Garnier, 1995). Interspecific differences in N growth response in C_3 grass species is reported primarily due to differences in the plasticity of net assimilation response to N supply (Taub, 2002). A previous field study showed N productivity varied among six hybrid poplars clones. However, the physiological factors that cause the variation have not been studied yet. In the present study, the growth response to N addition of two hybrid poplar clones and their N productivity were studied.

2.2 Review of steady state experimental technique

Plants require nutrients in increasingly larger amounts to maintain exponential growth characteristic of early growth. In traditional plant nutrition experiments, i.e. pot studies, only external nutrient solution concentration is maintained at a set level. The constant external concentration cannot satisfy the plant exponential growth needs, and the nutrient concentration in plants declines over time. Measurements of the plants are usually taken when the internal N concentration has dropped. Thus, the experiment becomes unrepeatable, and errors and complications cloud experimental conclusions. To solve this problem, Ingestad and Lund introduced the "steady-state" approach (Ingestad, 1979), which mimics plants growing in the field with an unlimited root volume. This approach supplies nutrients in an exponentially increasingly amounts over time to maintain a steady nutrient concentration within plants instead of maintaining a constant concentration in the nutrient solution. The increasing rate is quantified as 'relative addition rate' (RAR), i.e. amount of nutrient added per unit of time and per unit of nutrient already present in the plant. In a steady-state experiment, the RAR is the treatment variable to control plant growth, and nutrients are added at the same relative addition rates during experiment but with increasing absolute amounts per unit of time. The RAR strongly affects the plant nutrient uptake and growth, and they are described by two other dynamic variables: relative uptake rate (RUR) and relative growth rate (RGR). Relative uptake rate (g Ng N^{-1} day^{-1}) is the N uptake per unit weight of N in per unit of time. It can be calculated as:

$$RUR = (\frac{1}{n})(\frac{dN}{dt}) = \frac{\ln N_2 - \ln N_1}{t_2 - t_1} \quad (3)$$

Where N_1 and N_2 are the N contents on days t_1 and t_2, respectively.

Similarly, relative growth rate (g $g^{-1}day^{-1}$) is the biomass produced per unit weight of plant biomass in per unit of time. It can be calculated as follows:

$$RGR = (\frac{1}{W})(\frac{dW}{dt}) = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \quad (4)$$

where W_1 and W_2 are the plant weights on days t_1 and t_2, respectively.

When plants reach steady state, RUR equals RGR and is matched by RAR (Ingestad and Lund, 1986). A constant internal N concentration is achieved as well as the plant carbon allocation and RGR. RGR is positively correlated with plant N concentration until free access is reached. At this point, factors other than nutrition begin limiting plant growth. The steady state technique has been applied to tree nutrition studies for birch (*Betula verrucosa* Ehrh.) and grey alder(*Alnus incana* Moench) (Ingestad, 1981), poplar (*P. simonii* Carr.)(Jia, 1984), and aspen (*P.tremuloides* Michx.)(Coleman 1998), where the RAR was varied over a wide range. Plants grown with this technique attained a stable internal N concentration under a "constant N stress". Applying the steady-state technique has greatly improved clonal comparisons of nutrient studies by maintaining stable and comparable nutrient status in plants (Ingestad and Lund, 1986).

In most experiments that have used the steady-state technique, nutrients were added daily or hourly, through dripping irrigation, spray culture, or hydroponic system with computer control. The experimental setup is expensive and requires close monitor. In the present study, a lower frequency of addition was adopted. Whether plants could reach stable N concentration with low frequency nutrient addition was tested.

To understand the growth mechanism, RGR can be factorized into area based or dry weight based variables. When divided to area based variables, RGR is the product of leaf area ratio (LAR, leaf area per plant dry weight, $cm^2 g^{-1}$) and net assimilation rate (NAR, g $cm^{-2}d^{-1}$).

$$RGR = LAR \times NAR_{area} \quad (5)$$

The area-based NAR describes the dry weight production per unit leaf area per day, and photosynthesis per unit leaf area is its major component (Konings, 1990). RGR can be also divided into weight based variables as:

$$RGR = LWR \times NAR_{dw} \quad (6)$$

Where the dry weight based NAR_{dw} is the dry weight production per unit leaf dry weight (g g^{-1} day^{-1}), and LWR is the ratio of leaf weight to plant weight. NAR_{dw} can be converted from NAR_{area} as:

$$NAR_{dw} = NAR_{area} \times SLA \quad (7)$$

Where SLA is the specific leaf area (cm^2 g^{-1}), which reflects aspects of leaf thickness and/or density. It allows conversion of RGR calculation between the dry weight based and area based variables.

From equation (6) and (7), RGR is the product of LWR, SLA and NAR.

$$RGR = LWR \times SLA \times NAR_{area} \quad (8)$$

According to equation (8), the RGR response to the environment (N supply) or genetics (clone) can be dissected into LWR, SLA and NAR_{area}. To express the relative importance of each growth parameters, the concept of growth response coefficient (GRC) was introduced (Poorter and Nagel, 2000). The GRC is the relative change of a growth parameter with relative to the change in RGR. For example, the GRC of LWR can be calculated as:

$$GRC_{LWR} = \frac{\ln LWR_h - \ln LWR_l}{\ln RGR_h - \ln RGR_l} \quad (9)$$

where LWR_h, LWR_l, RGR_h and RGR_l represent the value of the variable at two treatment levels. Each of the GRC values indicates the relative contribution of that parameter to a change in RGR. A GRC value of 1 would indicate that the variation in RGR is related only to changes in LWR, while a value of 0 indicates that the changes in RGR are not related to LWR, but due to changes in SLA and/or NAR. The GRC values of the three components of RGR should sum to near 1. A GRC value can be higher than 1 if the increase in the growth parameter is stronger than the increase in RGR. If a GRC is lower than 0, it indicates an increase in that parameter goes with a decrease in RGR.

Research has shown that under nutrient rich conditions, interspecific differences in RGR were usually associated with differences in LAR (Taub, 2002). However, differences among species under N-limiting conditions were largely associated with NAR (Garnier, 1995). To our knowledge, few studies on

hybrid poplar growth response to N addition have been conducted. In the present study, clonal response to N addition was examined in hybrid poplars. The results may help improve our understanding of clonal behavior under field conditions.

2.3 Review of SPAD-502 chlorophyll meter application

The Minolta SPAD-502 meter was initially developed in Japan to diagnose N status of rice. Since then, its use has been quickly extended to other crops including corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), wheat (*Triticum aestivum* L.), strawberry (Fragaria × ananassa) and apple (*Malus domestica* Borkh). Most experiments showed good utility of the SPAD meter for predicting foliar N, but great variation exists, reportedly due to species, variety, leaf characteristics, growth stage and sample position.

Both linear and nonlinear relationships have been found between SPAD-502 meters and plant N concentration. Strong quadratic relations have been reported for eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.)(Moreau *et al.*, 2004) and corn (Wood, 1993), while linear equations best fit cottonwood (*Populus deltoides*)(Loh, 2002) and Peace Lily (*Spathiphyllum* Schott) (Wang, 2004).

These correlations are affected by species and cultivar. Different relationships between foliar N concentration and SPAD readings were observed in four hardwood species: sycamore (*Platanus occidentalis* L.), sweetgum (*Liquidambar styraciflua* L.), green ash (*Fraxinus pennsylvanica* Marsh.) and swamp cottonwood (*Populus heterophylla* L.) (Chang and Robison, 2003). Different relationships were also found between four apple cultivars (Neilsen *et al.*, 1995). Sampling season was reported to affect the relationship between SPAD-readings and N concentration in cottonwood (*Populus deltoides*) (Loh *et al.*, 2002) and apple (*Malus domestica* Borkh) (Neilsen *et al.*, 1995), possibly because of the change in non-chlorophyll related N partitioning. Other than chlorophyll variations, foliar water content (Chang and Robison, 2003) as well as leaf age (Coleman *et al.*, 1998) have been reported to impact the relationship among SPAD readings, chlorophyll content and foliar N concentrations.

Leaf thickness has been reported to affect SPAD readings. Excellent linear correlation between SPAD readings adjusted by leaf thickness and N concentration has been reported on tropical maize(Chapman, 1997, Peng, 1992) and sweetgum (*Liquidambar styraciflua* L.)(Chang, 2003). On the other hand, less significant relationships were also observed when considering the leaf thickness of eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.)(Moreau, 2004).

The experimental results from the studies show the feasibility of using the Minolta SPAD-502 on estimating N status, but great variations exist among the reports. A previous field experiment using the SPAD-502 meter on hybrid poplars demonstrated significant correlations between the SPAD values and hybrid poplar foliar N concentration, but the relationship varied by clone, N treatment and growth environment. In the present study, SPAD-502 meter was used to estimate hybrid poplar foliar N status and factors that affect the estimates were identified.

CHAPTER THREE

RESEARCH DESIGN AND METHODOLOGY

3.1 Research Objectives

The objective of first experiment was to examine the nitrogen use of 2 hybrid poplar clones identified in a previous field experiment to exhibit extreme N use. The present experiment was to further examine this observation and determine the physiological components that are responsible for the clonal response to N application.

The objective of the second experiment was to examine the use of SPAD-502 for estimating N status of hybrid poplar, by correlating the SPAD reading to leaf N concentration. The factors that affect the SPAD-N relationship were also examined.

3.2 Research design and methodology

The experiments were conducted in the greenhouse of WSU-Puyallup Research and Extension Center. Hybrid poplar clones GR-4284 (*Populus. deltoides* × *Populus. trichocarpa*) and 57-276 (*P. trichocarpa* × *P. deltoides*) were used as plant material in both experiments. Based on a field study of N use by six hybrid poplar clones, the two clones exhibited extreme field N use and were used as plant material in both experiments.

Greenwood cuttings were taken from trees in pots containing standard organic potting mixture. The cuttings were rooted under mist for two to three weeks before transplanted to experimental units. Uniform cuttings were then selected and transplanted into acid-washed pea gravel in plastic pots ($200cm^3$). The pots were then moved to a customized semi-hydroponic system in controlled temperature 20-22°C.

The semi-hydroponic system was used to grow the trees under two relative N addition rates. Eight pots were situated into a rectangular container (80cm × 50cm), constituting a growth unit, which was then connected to a large reservoir (approx. 7.5 gallon) of nutrient solution located below it. The nutrient solution was circulated into the growth unit every 30 minutes for 15 minutes by a timer-actuated submersible pump. When the pump shut off, the nutrient solution would drain back into the reservoir by

gravity (Fig.1). The cuttings were first grown in with deionized water without any nutrient for 2 weeks to dilute any internally stored N in each cutting.

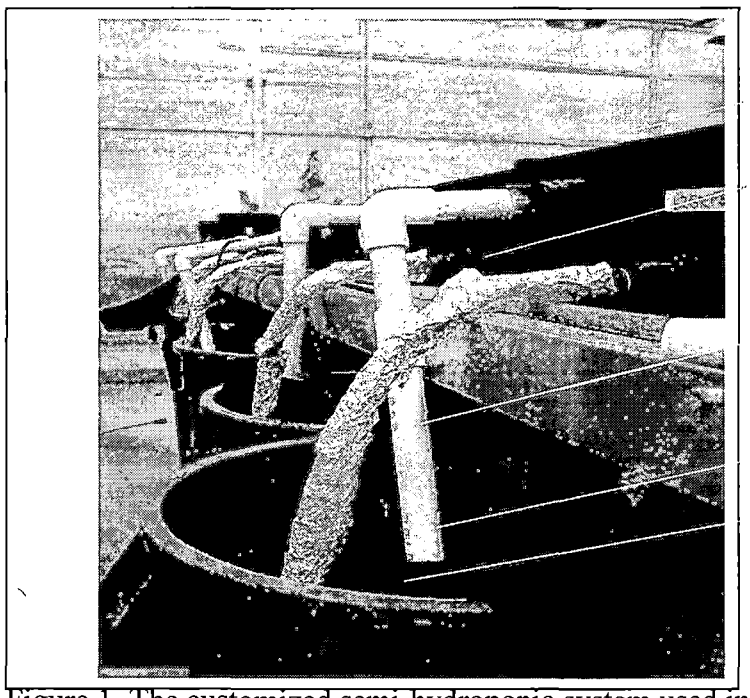

Plant growth unit
Nutrient solution transferring pipe wrapped with foil and connected to pump
Drainage pipe
Nutrient solution container cover
Nutrient solution container with pump inside

Figure 1. The customized semi-hydroponic system used in both experiments

3.2.1 Experiment 1 research materials and methods (Objective 1)

A randomized complete block design was used in experiment 1. The plants were randomly assigned into two blocks with two nitrogen treatments. There were sixteen plants in each clone-N treatments. The relative addition rates (RAR) of 1.5% and 10% were used to create low and high N treatment. Just before adding the first nutrient solution, the initial N amount in the cuttings was determined by measuring the dry weight and N concentration of a subset of extra cuttings. The N content was 9.2mg/per cutting and 73.6mg/growth unit (9.2 mg N × 8 cuttings). This was the starting N level with N and the other nutrients being added once a week at an exponentially increasing amount.

At the beginning of each week, the old solution was dumped and new nutrient was added to fresh deionized water. The amount of N added each week was the sum of the required N for the following seven days. The N needed each day was calculated from the initial N amount in the cuttings last day and the desired RAR, i.e., 1.5% and 10%.

$$A_{i+1} = A_i \times RAR \quad (8)$$

$$A = SUM(Ai) \quad i = 0, 1, 2, 3, 4, 5, 6 \quad (9)$$

Where A is the amount of N added per week. Ai is the amount of N required per day and i represents the day of the week. Figs.2a and b show the amount of N added for the 1.5% and10% RAR treatments, respectively.

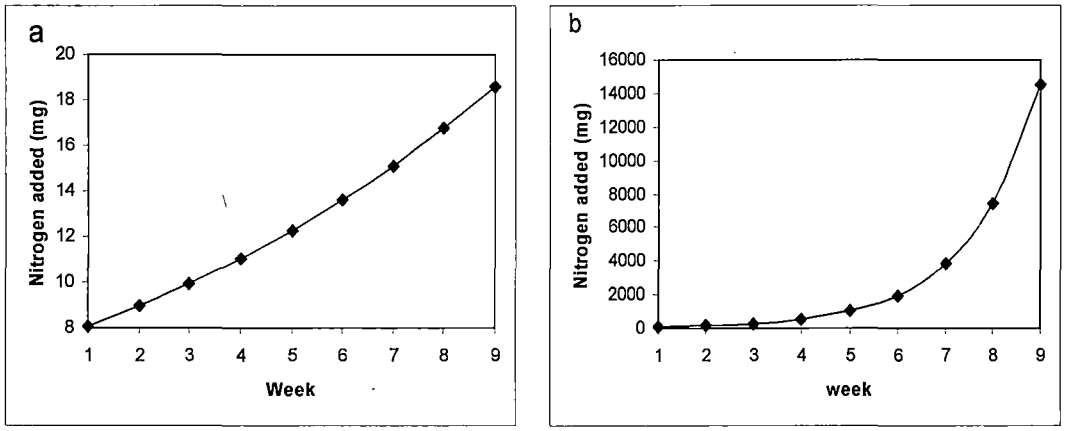


Figure 2. The amount of N added (mg) in (a) 1.5% RAR treatment (b) 10% RAR treatment over a 9 week period

Other nutrients were added at their optimum ratio to N according to Zabek (unpublished data): N:P:K:Ca:Mg:S:Fe:Mn:B:Zn:Cu:Mo:Mo:Na:Cl ratio of 100:80:15:45:20:9:0.7:0.4:0.2:0.06:0.03:0.007:0.03:0.003. A lag phase of 2 weeks existed between first adding nutrients and reaching the desired relative growth rate. Once steady-state was reached, 4 destructive harvests were done every 14 days. Two plants per growth unit were harvested at each time and plant growth parameters were measured. Leaf Plastochron Index (LPI) (Erickson, 1957) was used as the morphological time scale. First, a recently matured leaf (LPI 5-6) was sampled for photosynthetic rate measurement by TPS-1 photosynthesis system (PPsystems, Haverhill, MA). The number of branches and leaves were then counted. Leaf area was measured on fresh leaf samples with Agimage software (Decagon Instruments, Inc, Pullman, Washington). The samples were oven dried at 60°C for 60 hours, after which the roots, stems and leaves were weighed and recorded separately. These data were used to calculate relative growth rate (RGR) for each treatment and each clone. The dry plant components were ground and N concentration were measured in the leaves, stems and roots, respectively, with a Carbon-Nitrogen 2100 elemental analyzer (Carlo Erba Institute, Milan, Italy). Other physiological parameters, including net assimilation rate, biomass allocation and specific leaf area, were calculated based on these measurements.

3.2.2 Experiment 2 research materials and methods (Objective 2)

In experiment2, the potential for using the SPAD-502 to predict hybrid poplar N levels was determined. A semi-hydroponic system similar to experiment 1 was used. Clone 57-276 and GR-4284 were treated to relative addition rates (RAR) of 1.5% and 15% to create more contrasting N environments. During the steady-state phase, four harvests were made. At each harvest, one most recently mature leaf per plant (LPI 4-6) was sampled by taking 4 SPAD readings which were then averaged. Leaf area was measured with CI-203 laser area meter (CID, Inc., Vancouver, WA). Chlorophyll was extracted from a leaf punch (diameter=4.5mm) with 80% acetone and measured with spectrophotometer at 645 and 663nm(Arnon, 1949). The leaf was then dried. Dry weights and foliar N concentrations were measured as in experiment 1. The correlations among SPAD relative chlorophyll level, N and chlorophyll concentration were developed using SAS regression analysis. Chlorophyll to N ratio was calculated to examine the clonal effects on SPAD reading.

3.3 Statistical analysis

Statistical analysis was conducted using SAS software (SAS Institute, INC). In experiment 1, ANOVA was used to compare the effect of N addition rate on plant growth. In experiment 2, regression analysis was performed to test the linear relationships between the SPAD reading, N concentration and chlorophyll concentration. Significant P-level was 0.01 in both experiments.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Experiment 1 results and discussion

4.1.1 Steady-state test

The variation in plant internal N concentration was examined by harvests to see if steady state was achieved during the experiment. Constant plant N concentration was obtained with low variation over the harvests for each treatment during the experiment (Tab.1, Fig.3). No significant difference (P=0.12) was found in plant N concentration (PNC, mg g^{-1}) among the four harvests of each treatment. An insignificant PNC drop at last harvest was observed in the 10% RAR treatments (Fig. 3). This could be a result of insufficient uptake of the nutrient solution by plants. Better mixing may be required for more root contact and uptake of the nutrients at this RAR. The relative growth rate (RGR, g g^{-1} day^{-1}), relative uptake rate (RUR, g g^{-1}N day^{-1}) and relative addition rate (RAR, % g N^{-1} day^{-1}) were approximately equal to each other in each treatment, except at 1.5% RAR for GR-4284 (Tab.1). Because the 1.5% RAR treatment was a very low N addition rate, a small amount of N from other sources system could affect the actual amount of N added.

Table 1. Relative addition rate (RAR gN g N^{-1} day^{-1}), relative growth rate (RGR, g g^{-1} day^{-1}), relative uptake rate (RUR g g N^{-1} day^{-1}) and plant N concentration (PNC, mg g^{-1}) of clone 57-276 and GR-4284

clone	RAR	RGR	RUR	PNC
57-276	0.015	0.014	0.017	12.01 ± 1.03*
57-276	0.100	0.117	0.114	23.79 ± 1.78
GR-4284	0.015	0.031	0.026	12.27 ± 1.03
GR-4284	0.100	0.103	0.100	28.51 ± 1.53

*Standard deviation represents the variation among four harvesting dates

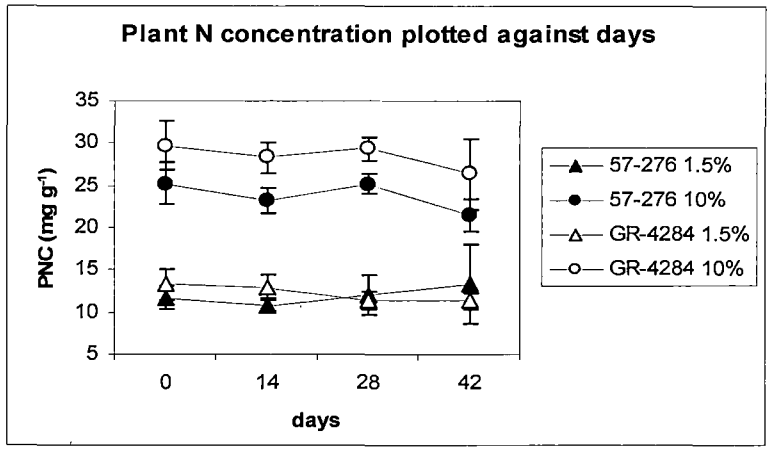


Figure 3. Plant N concentration (PNC, mg g^{-1}) of clone 57-276 and GR-4284 at each harvest date

Steady state growth also implies a constant root/shoot ratio (Ingestad, 1991). Such constancy was achieved in this study, as illustrated by Figs. 4. A strong linear correlation existed between the root and shoot dry weight at both N treatments ($P<0.01$), indicating a stable biomass allocation during the experimental period. The regression models for each treatment and clone are shown in Tab.2.

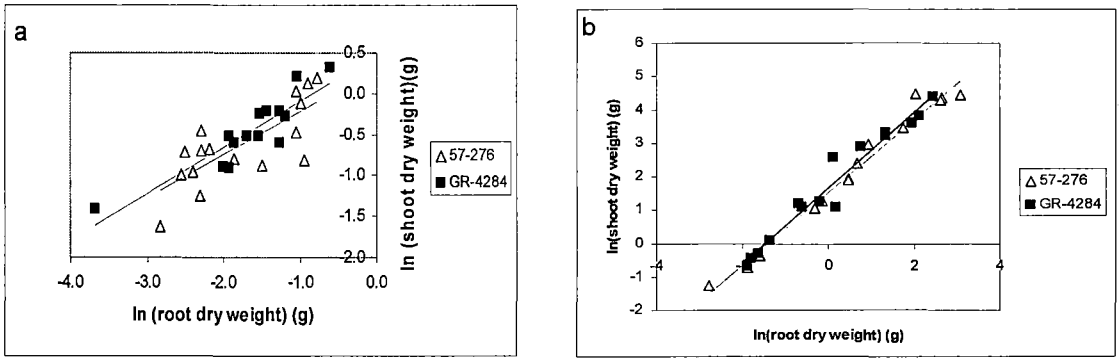


Figure 4. The allometric relationships between the shoot and root dry weight of clones 57-276 and GR-4284 grown at (a)1.5% RAR and (b) 10% RAR

Table 2. The regression coefficients of shoot-root relationship for clones 57-276 and GR-4284 at 1.5% and 10% RAR. The model was: Ln(shoot)=slope×Ln(root) + intercept

Clone	RAR	Slope	Intercept	R^2
57-276	0.015	0.53	0.32	0.59
57-276	0.100	1.09	1.54	0.98
GR-4284	0.015	0.57	0.47	0.80
GR-4284	0.100	1.13	1.65	0.96

The experimental results showed that constant internal N concentration was achieved, indicating that the trees were at steady-state. Thus, using a low frequency (once a week) of adding nutrient solutions

can be used for steady - state nutrient studies. Improved mixing of the nutrient solution may solve the observed reduction in internal PNC at high RAR.

4.1.2 Plant growth response to N treatments

Plant growth response to N was affected by clone (Fig. 5). Biomass was unaffected by RAR or clone at the beginning of the experiment. This trend changed at the second and subsequent harvests. At each harvest, the dry weight of 57-276 was lower than GR-4284 at low RAR and higher than GR-4284 at high RAR, with greater difference at high RAR (Tab. 3). The difference increased with time and was significant by the fourth harvest in high RAR treatment (Tab. 4). The clonal difference in plant biomass suggests that GR-4284 may be better adapted to low N environment, while 57-276 showed greater growth response than GR-4284 at high N.

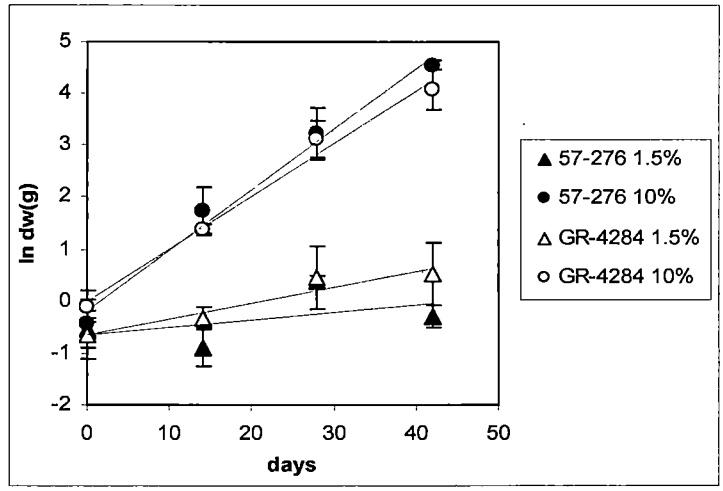


Figure 5. Total plant dry weight (ln dw) accumulation over time for clones 57-276 and GR-4284 grown at two different relative addition rates (RAR). The ln(dw) drop at 2nd and 4th harvest of 1.5% treatments was due to variability in plant size

Table 3. Plant dry weight (g) of clones 57-276 and GR-4284 at each harvest

Treatment	Plant Dry Weight (g)			
	Harvest1	Harvest2	Harvest3	Harvest4
57-276 1.5%	0.60	0.42	1.46	0.76
57-276 10%	0.70	6.13	27.2	94.16**
GR-4284 1.5%	0.56	0.74	1.81	1.95
GR-4284 10%	0.94	3.95	23.64	62.43

** Significant difference existed between 57-276 and GR-4284 at same RAR level($P<0.01$)

Table 4. Probability of biomass differences between clones 57-276 and GR-4284 at each harvest

RAR	P value of the biomass difference between clones

	Day 0	Day 14	Day 28	Day 42
1.5%	0.99	0.95	0.94	0.80
10%	0.96	0.65	0.46	<0.0001

Relative Growth Rate

The relative growth rate (RGR, g g^{-1} day^{-1}) of each clone at each RAR level was calculated as the slopes of the regression lines in Fig 5. The RGR of both clones increased significantly from low to high RAR ($P<0.01$) (Fig.6). The RGR of 57-276 increased more than 8 times when RAR increased from 1.5% to 10%, and the RGR of GR-4284 more than tripled. When compared at same RAR or plant N concentration, the RGR of GR-4284 tended to be higher at low RAR, but lower than 57-276 at high RAR (Fig. 6). The RGR of the two clones appear to be similar at an RAR of around 6%. This indicated that while severely limited in the relative growth rate at low N, clone 57-276 responded better to N addition than GR-4284.

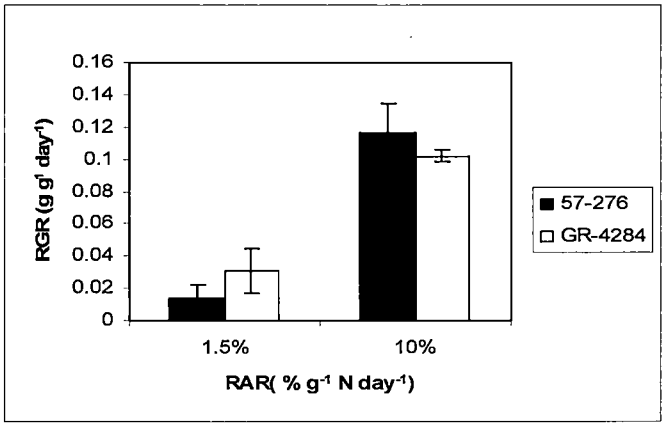


Figure 6. Relative growth rate (RGR, g g^{-1} day^{-1}) of clones 57-276 and GR-4284 at 1.5% and 10% relative addition rates.

Growth Variables Analysis

RGR is defined as the product of net assimilation rate (NAR, g cm^{-2} day^{-1}), specific leaf area (SLA, cm^2 g^{-1}) and leaf weight ratio (LWR), as

$$RGR = NAR \times SLA \times LWR \quad (10)$$

Net assimilation rate describes the amount of plant dry weight gained per unit of leaf area per day. Specific leaf area is the ratio of leaf area to leaf dry weight, and it has been used to describe the leaf thickness and/or leaf density. Leaf weight ratio describes the biomass allocation to leaves relative to the rest of the plant.

The NAR of 57-276 and GR-4284 responded differently to increasing N (Fig. 7). The NAR of 57-276 increased significantly from 0.0003 to 0.0011 g cm^{-2} day^{-1} in response to N addition ($P<0.01$). In contrast, NAR of GR-4284 didn't change significantly ($P=0.17$), increasing from 0.0005 to 0.0008 g cm^{-2} day^{-1}. LWR and SLA of both clones increased significantly from low to high RAR ($P<0.01$) (Figs.8 & 9).

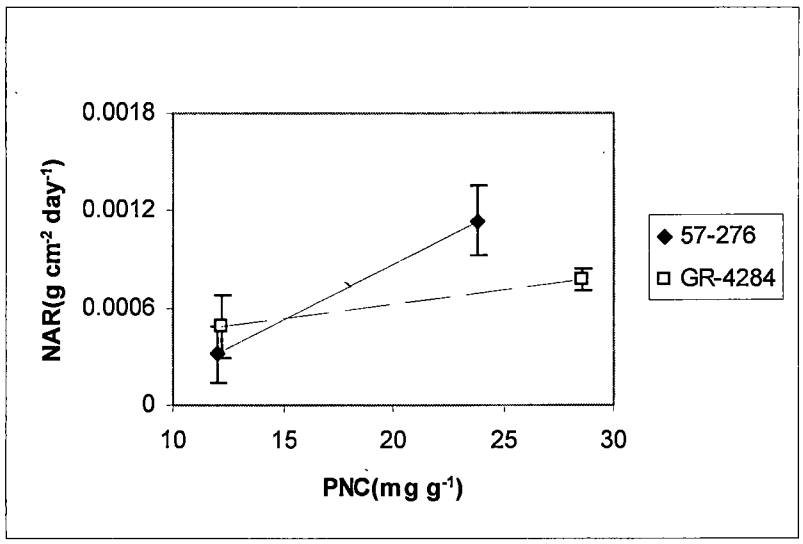


Figure 7. Net assimilation rate (NAR, g cm^{-2} day^{-1}) of clones 57-276 and GR-4284 at different plant N concentration

–: difference between 1.5% and 10% RAR treatment is significant ($P<0.01$)

---: difference between 1.5% and 10% RAR treatment is not significant

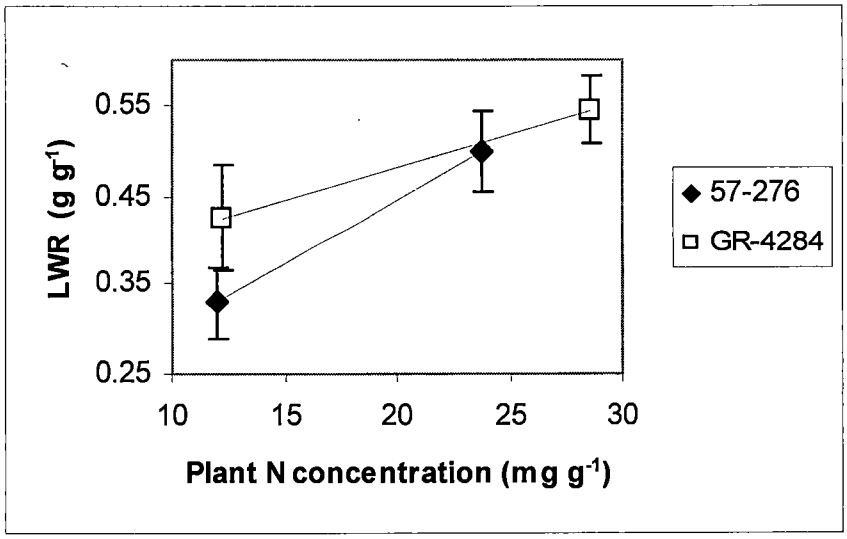


Figure 8. Leaf weight ratio (LWR) of clones 57-276 and GR-4284 at low and high leaf N concentration

—: difference between 1.5% and 10% RAR treatment is significant ($P<0.01$)

---: difference between 1.5% and 10% RAR treatment is not significant

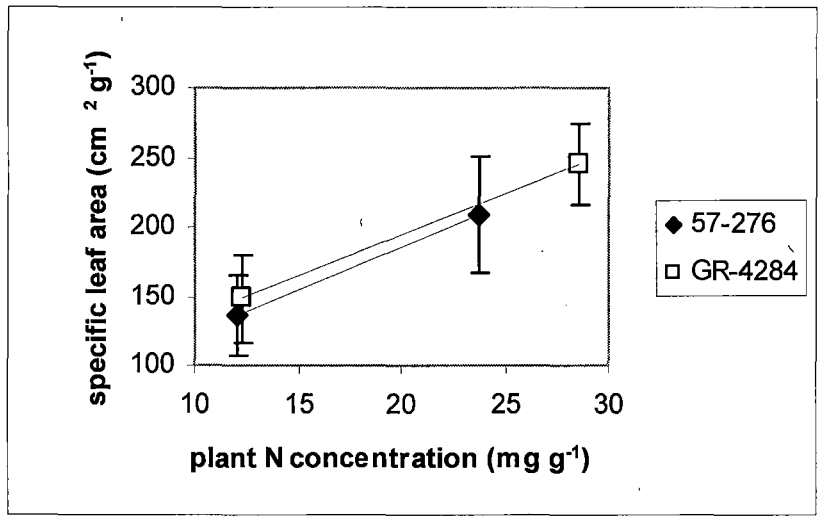


Figure 9. Specific leaf area (SLA, $cm^2\ g^{-1}$) of clones 57-276 and GR-4284 at low and high leaf N concentration
—: difference between 1.5% and 10% RAR treatment is significant ($P<0.01$)
---: difference between 1.5% and 10% RAR treatment is not significant

According to Equation 10, the change in RGR can be directly related to the changes in NAR, SLA and LWR. But what is the relative importance of each variable in changing the relative growth rate? Do they contribute similarly to the RGR increase with N addition? Are the patterns same or different for the two clones? To answer these questions, the contribution of the NAR, SLA and LWR to the changes in RGR with N addition was examined with the Growth Response Coefficient (GRC) method of Poorter and van der Werf (1998).

The patterns of GRCs were affected by clone (Fig.10). For 57-276, GRC was NAR =0.61, LWR=0.2 and SLA =0.2. For GR-4284, the trend in GRC was SLA =0.42, NAR =0.37 and LWR =0.2. The differences observed in GRC suggest that the mechanism of RGR increase in response to N addition differed between the two clones. For 57-276, the increase in RGR was primarily due to an increase in NAR, with SLA and LWR contributing to the same relatively low extent. For GR-4284, the increase in RGR was a result of changes in SLA and NAR with LWR contributing the least. GRC_{LWR} was same for the clones, which indicated the biomass allocation to leaves made similar contribution to RGR increase. SLA and LWR can be combined to leaf area ratio (LAR $cm^2\ g^{-1}$) as:

$$LAR = SLA \times LWR \quad (11)$$

LAR has been used to describe the area of a plant's assimilatory material of to the total weight of the plant. The GRC of LAR is the sum of GRC_{SLA} and GRC_{LWR}:

$$GRC_{LAR} = GRC_{SLA} + GRC_{LWR} \quad (12)$$

For 57-276, the GRC_{NAR} was still higher than GRC_{LAR} (GRC_{LAR} = 0.4), but lower in GR-4284 (Fig.10). These contrasts revealed the different ways the clones increased their growth rates when exposed to increasing N availability. The growth increase of 57-276 was more dependent on increasing productivity per unit leaf area, while GR-4284 was more dependent on leaf area expansion. Researches have shown that interspecific differences in growth response to N were primarily associated with species differences in the response of NAR (Taub, 2002). The greater growth response of 57-276 to N was probably due to a more effective increase in NAR.

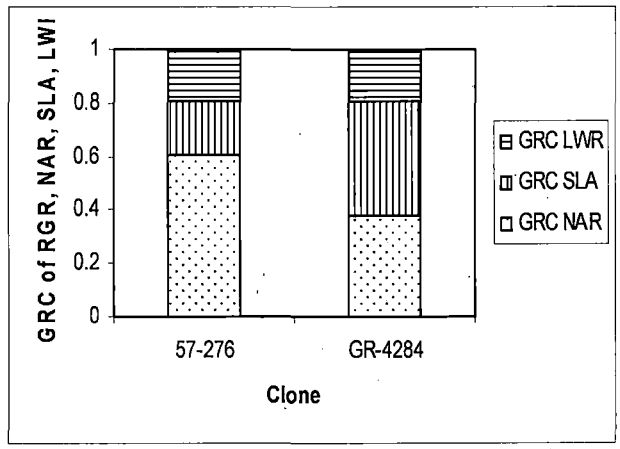


Figure 10. The GRC_{LWR}, GRC_{SLA}, GRC_{LAR} and GRC_{NAR} of clones 57-276 and GR-4284

Photosynthesis rate

Because photosynthesis is a major component of NAR, the photosynthesis rate (A, μmol CO_2 m^{-2} s^{-1}) of each clone was measured. The photosynthesis rate of both clones increased significantly from low to high N treatment ($P<0.01$) (Fig. 11). It increased almost 4-fold from 3.4 to 13.0μmol CO_2 m^{-2} s^{-1} for 57-276, and by 2.7-fold, from 4.8 to 12.8μmol CO_2 m^{-2} s^{-1} for GR-4284. No clone-RAR interaction was found ($P=0.69$). The photosynthesis rate based on leaf dry weight (μmol CO_2 g^{-1} s^{-1}) showed the same trend ($P=0.76$), indicating that both clones responded similarly to increasing N. So the question is, with similar increase in A, why did the NAR of 57-276 and GR-4284 increase differently?

Research has shown that NAR increases with PNC, but with diminishing returns, and saturates at certain PNC (Hirose, 1988). The NAR of clone GR-4284 appeared to be nearly saturated in the PNC range of this study, while 57-276 still increased. NAR is considered an integrative measure of photosynthesis over time that also includes dark respiration, while A is an instantaneous measure of carbon dioxide exchange. Although the degree of A increase was similar between the clones, GR-4284 may exhibit a higher respiration rate with increasing N addition compared to 57-276. Therefore, a similar A increase would not necessarily lead to a similar increase in NAR.

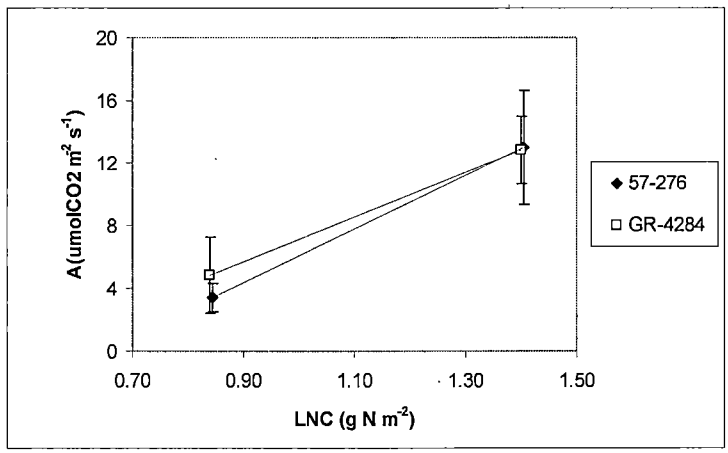


Figure 11. Photosynthetic rate (A μmol CO_2 m^{-2} s^{-1}) of 57-276 and GR-4284 at two levels of leaf N concentration (gN m^{-2})

–: difference between 1.5% and 10% RAR treatment is significant ($P<0.01$)

---: difference between 1.5% and 10% RAR treatment is not significant

Conclusions in plant growth response to increased N addition

1) RGR of both clones increased with N addition rate, as a result of increases in leaf area and/or productivity per unit leaf area.

2) There was clonal difference in the growth response to N. Compared to GR-4284, 57-276 was more sensitive to N at low N than GR-4284, and was more responsive as N increased. GR-4284 was more tolerant of low N, but was less responsive at higher N.

3) Clones exhibited different mechanisms by which RGR increase when exposed to high N. 57-276 increased productivity per unit leaf area, whereas GR-4284 relied on leaf expansion.

4) There was no clonal difference in photosynthetic response to N application.

4.1.3 N productivity and plant N concentration

To understand N use, relative growth rate can be partitioned into N productivity (Pn, g plant gN^{-1} day^{-1}) and plant N concentration (PNC, mg g^{-1}), according to Ingestad (1979):

$$RGR = Pn \times PNC \quad (13)$$

Where Pn is the the amount of plant dry weight gained per unit of N per day. Variations in Pn and PNC are important in determining the relative growth rate.

The response of Pn to N addition was affected by clone (Fig. 12). Pn of 57-276 increased significantly ($P<0.01$) from 1.2 to 4.9 g plant g N^{-1} day^{-1} with increasing RAR, a 4-fold increase. However, this was not the case for GR-4284 ($P=0.22$), where Pn increased from 2.5 to 3.6 g plant g N^{-1} day^{-1}. No clone and RAR interaction was observed ($P=0.07$).

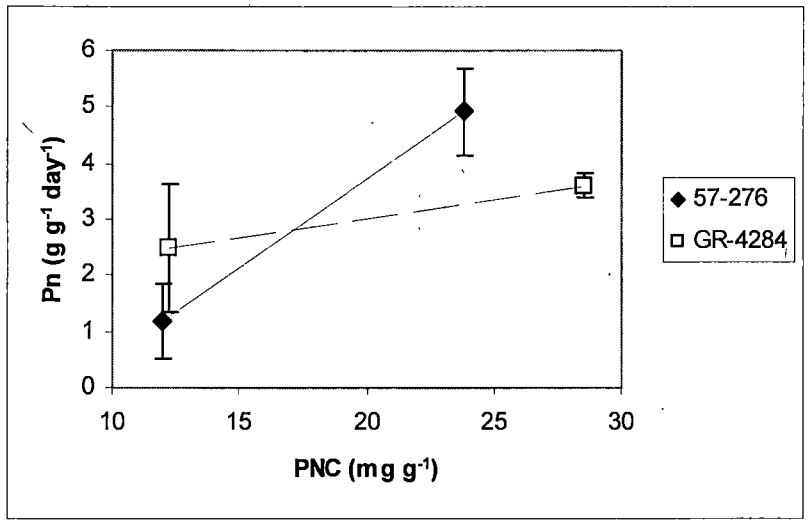


Figure 12. N productivity (Pn, g plant gN^{-1} day^{-1}) of clones 57-276 and GR-4284 at two plant N concentrations

–: difference between 1.5% and 10% RAR treatment is significant ($P<0.01$)

---: difference between 1.5% and 10% RAR treatment is not significant

PNC increased significantly with increasing RAR ($P<0.01$) for both clones (Fig. 13). PNC of 57-276 increased 98.1% from low to high RAR. The increase for GR-4284 PNC was 131.8%. The interaction of clone and RAR was significant ($P<0.01$).

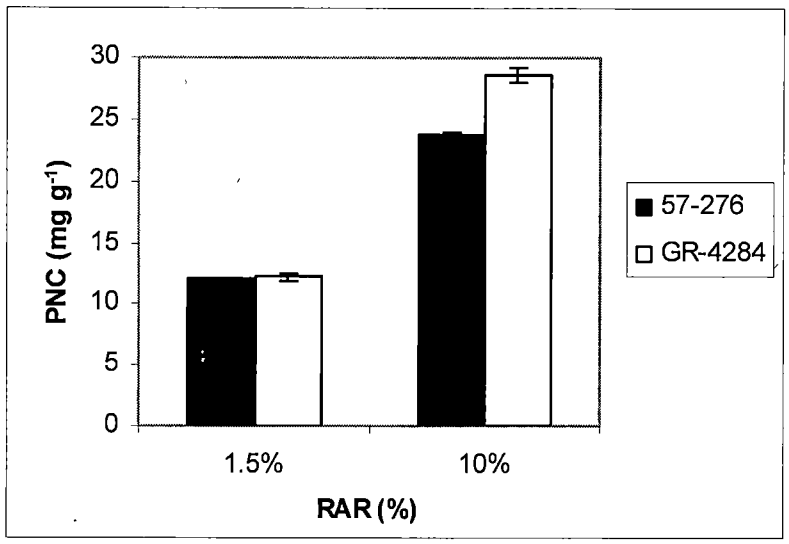
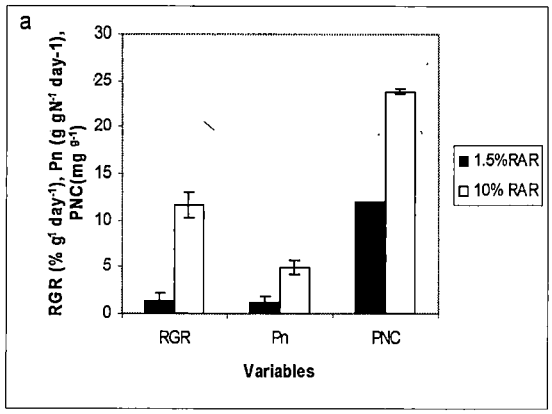


Figure 13. Plant N concentration (PNC mg g^{-1}) of clones 57-276 and GR-4284 at two relative addition rates (RAR % gN^{-1} day^{-1})

Contributions of PNC and Pn to RGR increase with N addition

According to Equation 13, the increase of RGR depends on the change of PNC and Pn. The RGR increase of 57-276 resulted from the increase in Pn and PNC as they both increased significantly (Fig.14a). The RGR increase of GR-4284 was mainly the result of an increase in PNC, since Pn did not increase significantly (Fig.14b).

The contribution of the growth response coefficients confirmed the observed clonal patterns (Fig. 15). The increase in RGR was the result of an increase primarily Pn for 57-276, but was the result of plant N concentration for GR-4284. By increasing the productivity per unit of N, the RGR increased more effectively for 57-276 than GR-4284.



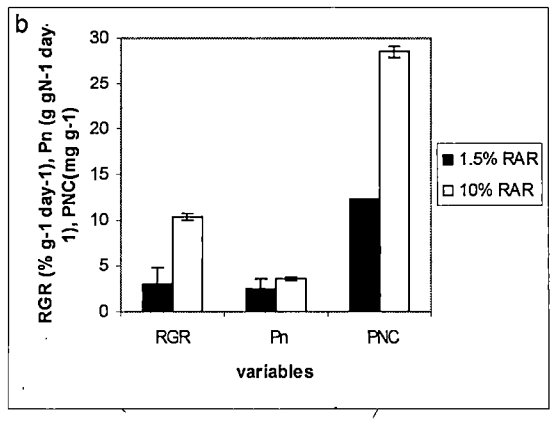


Figure 14. Relative growth rate (RGR, % g^{-1} day^{-1}), plant N concentration (PNC, mg g^{-1}) and N productivity (Pn g gN^{-1} day^{-1}) of clones (a) 57-276 at 1.5% and 10% RAR and (b) GR-4284 at 1.5% and 10% RAR

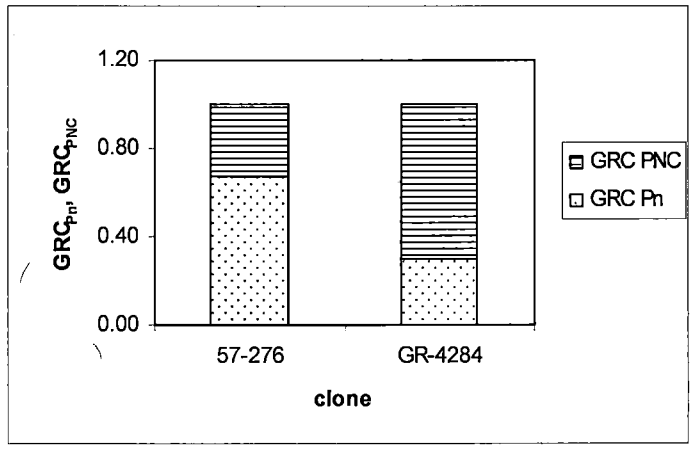


Figure 15. Growth Response Coefficient of N productivity (Pn g gN^{-1} day^{-1}) and plant N concentration (PNC mg g^{-1}) for clones 57-276 and GR-4284

Leaf N ratio and leaf N productivity

N productivity can be determined by leaf N productivity (Pnl, g g leaf N^{-1} day^{-1}) and leaf N ratio (LNR) as

$$Pn = Pnl \times LNR \qquad (14)$$

Where Pnl is the biomass gained per unit of leaf N per day and LNR is the ratio of leaf N content to plant N content. Clone and N treatment had different effects on Pnl (Fig. 16). Pnl increased significantly from 3.7 to 8.1 g gN^{-1} day^{-1} for 57-276 ($P<0.01$), but remained essentially the same for GR-4284, 5.7 to 5.5 g gN^{-1} day^{-1}($P=0.22$). For LNR, both clones increased significantly from low to high RAR ($P<0.01$), with 57-276 increasing from 0.32 to 0.59 and GR-4284 from 0.42 to 0.64 (Fig. 17). The results indicated that Pn increased in 57-276 by increasing in both leaf N productivity and leaf N ratio, while the small increase in the Pn of GR-4284 was largely caused by increasing N amounts in leaves, rather than by the productivity per unit leaf N.

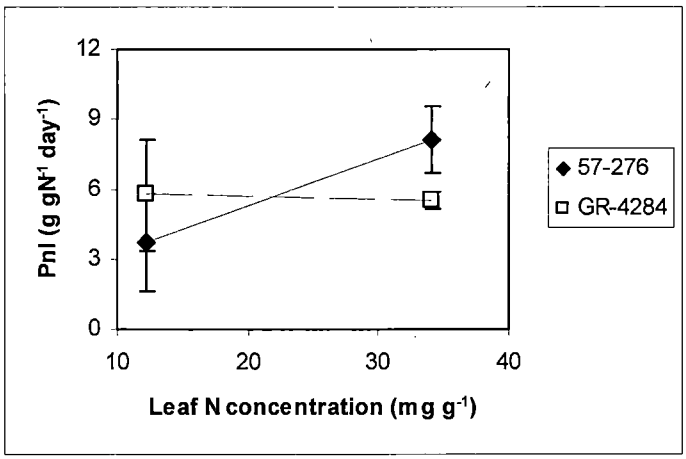


Figure 16. Leaf N productivity (Pnl, g g leaf N^{-1} day^{-1}) of clones 57-276 and GR-4284 at two plant N concentrations

–: difference between 1.5% and 10% RAR treatment is significant (P<0.01)

---: difference between 1.5% and 10% RAR treatment is not significant

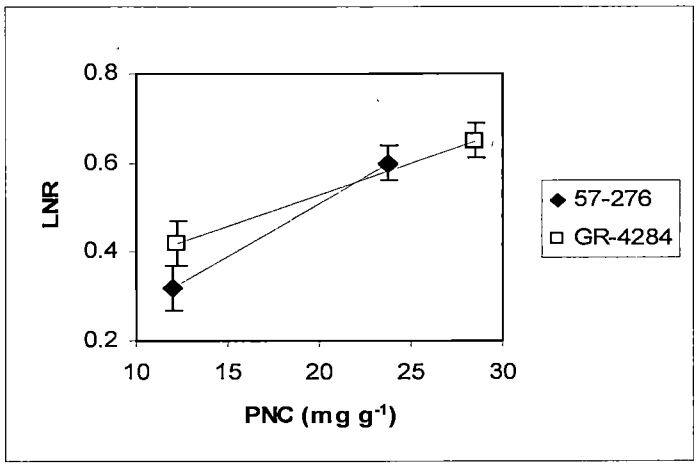


Figure 17. Leaf N ratio (LNR) of clones 57-276 and GR-4284 at two plant N concentrations

–: difference between 1.5% and 10% RAR treatment is significant (P<0.01)

---: difference between 1.5% and 10% RAR treatment is not significant

Photosynthetic N use efficiency

Leaf N productivity has been reported to be closely associated with photosynthetic N use efficiency (NUE_{PH}, μmol CO_2 gN^{-1} s^{-1}) (Garnier et al, 1995). NUE_{PH} is defined as the ratio between the rate of photosynthesis (A, μmol CO_2 m^{-2} s^{-1}) and leaf N concentration (mg cm^{-2}).

The NUE_{PH} of both clones increased significantly from low to high RAR (P<0.01), and no clone-RAR interaction was found (P=0.56) (Fig.18). The NUE_{PH} of 57-276 increased from 3.8 to 9.8 μmol CO_2 g N^{-1} s^{-1}, and GR-4284 increased from 5.1 to 9.7 μmol CO_2 g N^{-1} s^{-1}. This indicated the response of

photosynthetic N use efficiency to N addition didn't differ between clones. Therefore, the variation of leaf N productivity response to N addition was probably not due to the ability of carbon fixation per unit of N, but due to the variation in respiration loss per unit of N. The respiration rate per unit N of GR-4284 may have increased faster than that of 57-276 with N addition and so the net gain per unit leaf N of GR-4284 didn't increase as much as 57-276.

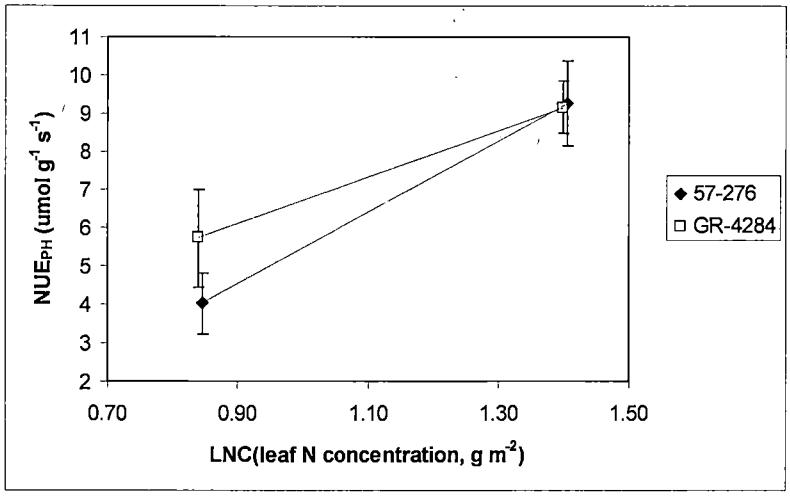


Figure 18. Photosynthetic N use efficiency (NUE_{PH}, μmol CO_2 gN^{-1} s^{-1}) of 57-276 and GR-4284 at two leaf N concentrations (LNC, g m^{-2})

–: difference between 1.5% and 10% RAR treatment is significant ($P<0.01$)

---: difference between 1.5% and 10% RAR treatment is not significant

Conclusions in N use response to N addition

1) When external N changes from low to high level, there was clonal difference in the mechanism of the growth response to the added N. 57-276 depended more on increasing the productivity per unit of N, and GR-4284 increased the amount of N present in the plant, especially in leaves, to achieve growth rate increase as N addition increased.

2) There was clonal difference in the response of N productivity to N addition range studied. The productivity per unit of N in 57-276 increased more effectively by changing the leaf N productivity with N addition. Neither the leaf N nor the N productivity of GR-4284 responded to the N addition.

3) There was no clonal difference in the response of photosynthetic N use efficiency to N addition.

4.2 Experiment 2 results and discussion

The Minolta SPAD-502 was designed to estimate leaf chlorophyll level, and therefore, SPAD values were first correlated with acetone-extracted chlorophyll concentrations, and the SPAD-chlorophyll

relationship of each clone was examined. The chlorophyll concentrations were then related to foliar N concentrations to check the chlorophyll-N relation for each clone. Finally, the SPAD values were correlated to N concentration. Examining the interrelationship of the three variables will help to determine factors affecting SPAD reading used for estimating leaf N.

The clone and N effects on SPAD, chlorophyll and foliar N concentration

In both clones, 15% RAR treatment significantly increased foliar N concentration, chlorophyll concentration and SPAD readings ($P<0.01$) (Fig.19). Across RAR treatments, SPAD values ranged from 17.5 to 37.7. Chlorophyll concentration ranged from to 1.81 to 13.09 mg g^{-1}, and foliar N concentrations ranged from 13.4 to 50.2 mg g^{-1}.

In comparison to the low RAR treatment, the high RAR treatment raised foliar N by 186% and chlorophyll concentration by 218% in 57-276. The increases for GR-4284, were 68% and 192%, respectively.

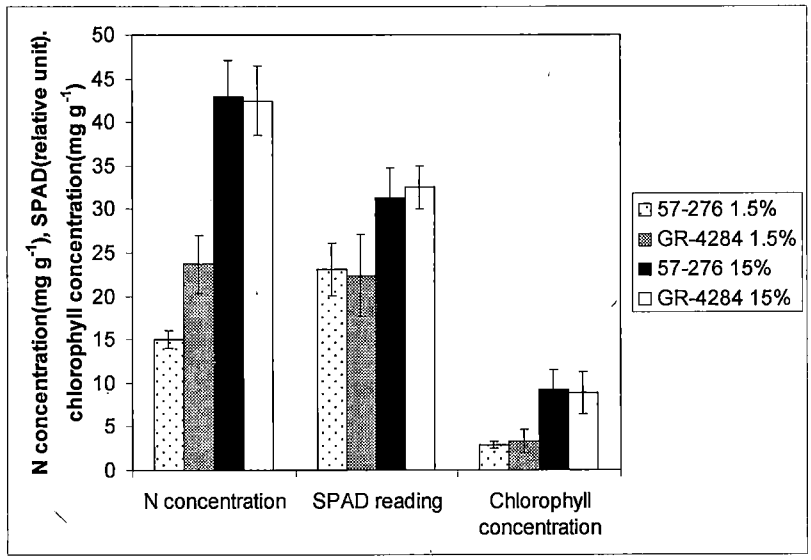


Figure 19. Effects of two N relative addition rate on leaf N concentration (mg g^{-1}), SPAD values and chlorophyll concentration (mg g^{-1}) of two hybrid poplar clones, GR-4284 and 57-276 The means and standard errors of 18 samples for clone 57-276 and 9 samples for clone GR-4284 are shown

Relationship of SPAD readings and chlorophyll

SPAD readings exhibited a significant positive linear correlation with acetone-extracted chlorophyll concentrations for both clones (Tab.5, Fig. 20). The slope was 1.23 for 57-276 and 1.60 for GR-4284. The slopes were not significantly different ($P=0.21$), suggesting that the SPAD prediction of chlorophyll

concentration is independent of clones. However, 57-276 showed a slightly better correlation than GR-4284 (Tab.5).

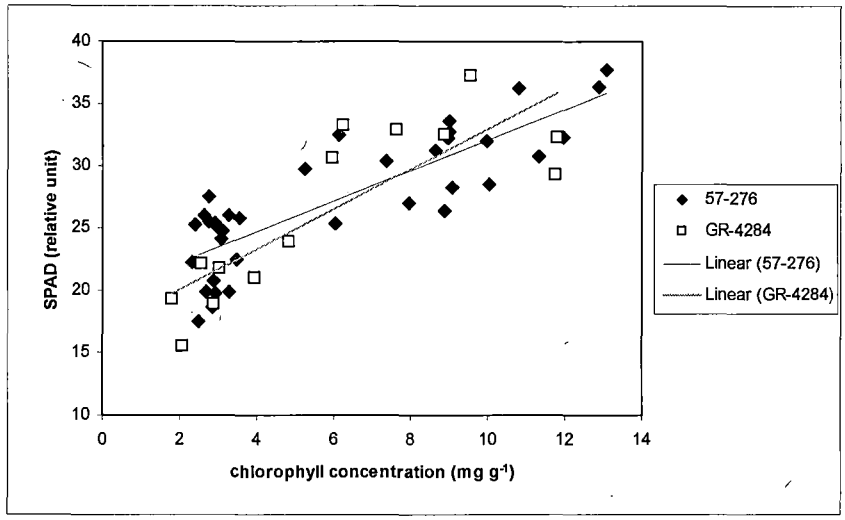


Figure 20. The relationship between SPAD and acetone-extracted chlorophyll concentration for two hybrid poplar clones, 57-276 and GR-4284

Table 5. Linear correlation between SPAD and acetone-extracted chlorophyll concentration for clone 57-276, GR-4284 and their combination

Clone	Model	P value	R^2
57-276	SPAD =1.23chl + 19.77	<0.01	0.69
GR-4284	SPAD =1.60chl + 17.1	<0.01	0.66
Combined	SPAD =1.33chl + 19.03	<0.01	0.68

Relationship of chlorophyll and foliar N concentration

Significant linear relationships were found between foliar N and chlorophyll concentration in both clones (Tab.6, Fig.21). The slopes were significantly different between the clones ($P<0.05$). The slope was 3.77 for 57-276 and 2.75 for GR-4284. At the same N concentration level, 57-276 had higher chlorophyll concentration than GR-4284 until the N concentration reached 45 mg g^{-1} (Fig. 21), indicating 57-276 allocated greater amounts of N into chlorophyll production when N was limiting. The chlorophyll concentration to N concentration ratio (Chl/N) also supported this interpretation (Fig.22). Clone 57-276 had significantly higher Chl/N ratio at low N treatment ($P<0.01$). At high N treatment, the difference was not significant ($P=0.71$). The Chl/N ratio and the chlorophyll-N regression model suggest that N allocation to chlorophyll is under clonal control and varies between clones.

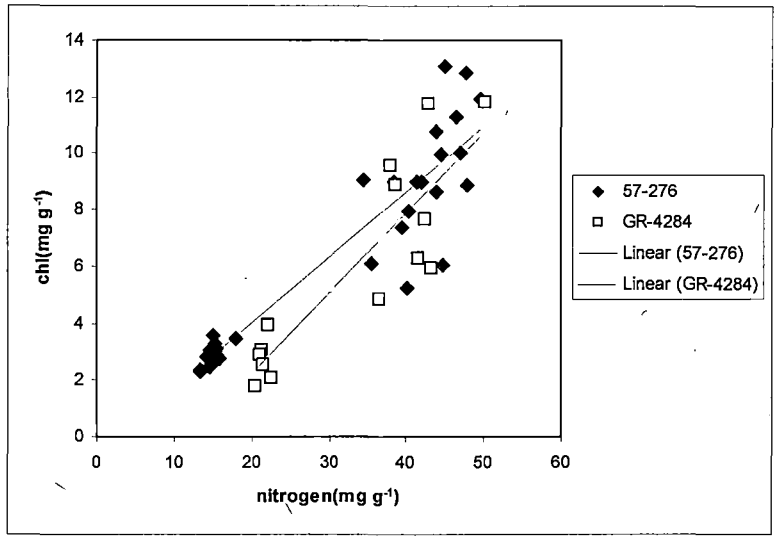


Figure 21.The relationship between foliar N concentration (mg g^{-1}) and acetone-extracted chlorophyll concentration (mg g^{-1}) for two hybrid poplar clones, 57-276 and GR-4284

Table 6. Linear regression model between foliar N concentration and acetone-extracted chlorophyll concentration for two hybrid poplar clones, 57-276 and GR-4284

Clone	Model	P value	R^2
57-276	chl = 0.23N - 0.59	<0.01	0.87
GR-4284	chl = 0.28N – 3.38	<0.01	0.77

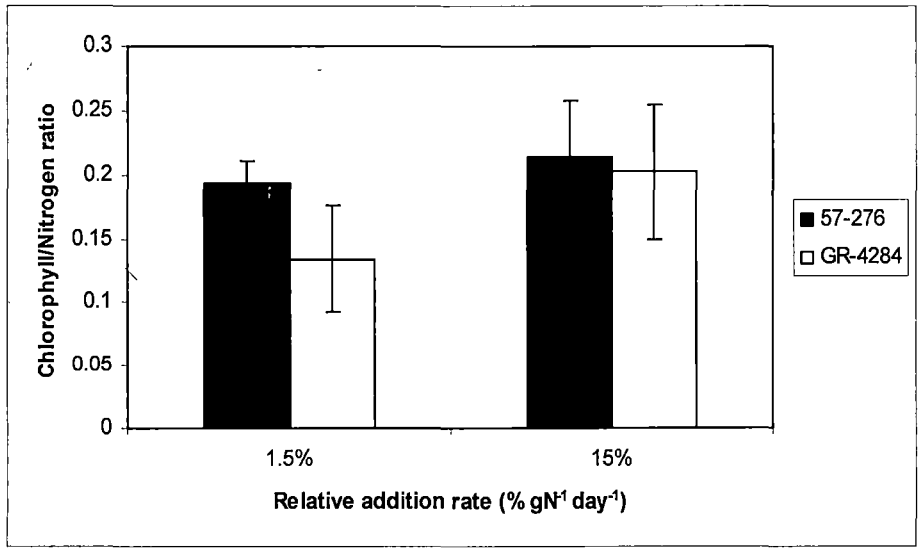


Figure 22. Ratio of chlorophyll to N (chl/N) as influenced by clone and relative addition rate

Relationship of SPAD readings and foliar N concentration

The linear relationship between SPAD values and foliar N concentrations was significant for both 57-276 and GR-4284, and their slopes were significantly different (Fig.23, Tab.7). The regression lines

crossed at 40.5 mg g^{-1}, meaning that below this level, the SPAD meter gave a higher value in clone 57-276 compared to GR-4284 at a given N level. When N concentration was above this level, the SPAD reading of clone 57-276 was lower than GR-4284.

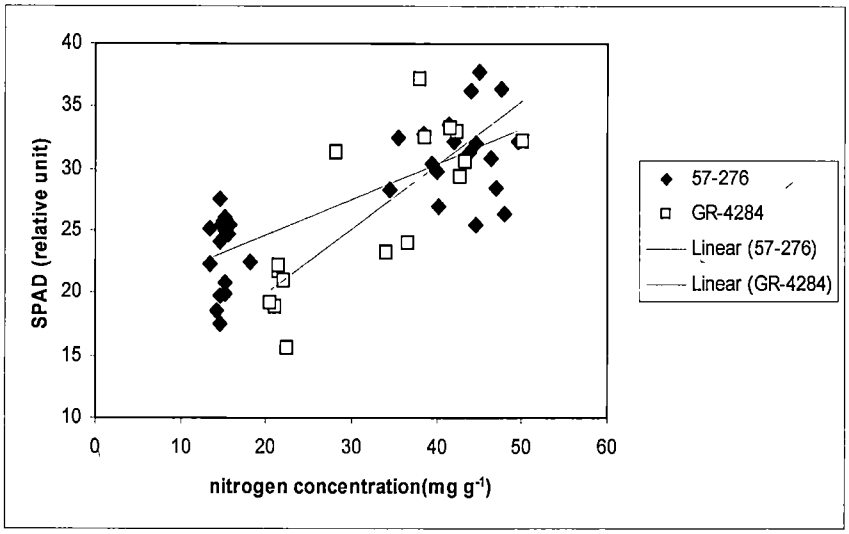


Figure 23. The relationship between SPAD value and foliar N concentration (mg g^{-1}) for two hybrid poplar clones, 57-276 and GR-4284

Table 7. Linear regression model between SPAD values and foliar N concentration for two hybrid poplar clones,57-276 and GR-4284

Clone	Model	P value	R^2
57-276	SPAD = 0.28N + 18.99	<0.0001	0.61
GR-4284	SPAD = 0.53N + 10.02	<0.0001	0.68

Clone 57-276 showed better SPAD - chlorophyll and N-chlorophyll relationships than GR-4284, however when it came to SPAD-N correlation, the R^2 for 57-276 was lower than GR-4284. The R^2 values indicated factors other than N and chlorophyll concentration impact SPAD meter readings.

Previous studies have reported that SPAD readings can be affected by leaf thickness. To explore this effect in hybrid poplar, specific leaf area (SLA, $cm^2 g^{-1}$) was used as an indicator of leaf thickness. SLA is defined as leaf area per unit of leaf dry mass, and accordingly, leaf thickness decreases as SLA increases. A leaf with high SLA has large area for given mass.

Leaf samples of 57-276 with similar N concentrations were selected and their SPAD readings were plotted against their SLA values (Figs.24 a and b). SPAD readings were found to be negatively correlated with SLA (Tab.8). The acetone-extracted chlorophyll didn't show such a negative relationship

with SLA, suggesting that the relationships between SPAD values and SLA was not due to changes in chlorophyll, but to the way SPAD-502 meter works. As a leaf thickens, less light is transmitted through the leaf by the SPAD, resulting in a higher value.

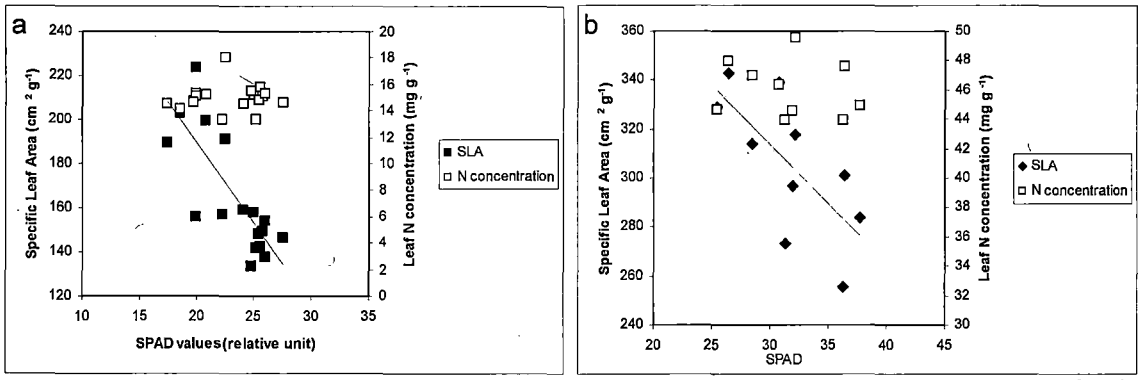


Figure 24. The relationship between SPAD readings and specific leaf area (SLA, $cm^2\ g^{-1}$) of selected leaves with similar N concentration in clone 57-276 at (a)1.5% RAR and (b) 15% RAR

Table 8. Linear correlation between SPAD values and specific leaf area for selected leaves with similar N concentration of clone 57-276 at two RAR levels

Treatment	Model	P value	R^2
57-276 1.5%	SPAD = -0.09SLA + 37.47	<0.01	0.60
57-276 15%	SPAD = -0.12SLA + 70.17	=0.01	0.56

In general, GR-4284 tended to have higher SLA than 57-276 at both N treatments (Fig. 25). At 1.5% RAR, the SLA of GR-4284 was 12% higher than that of 57-276. When leaves of the two clones had the similarly low N concentration, the thinner leaves of GR-4284 (higher SLA) resulted in lower SPAD readings. Under high N, the SLA of both clones increased to similar levels, eliminating clonal differences in the SPAD readings (Fig.25).

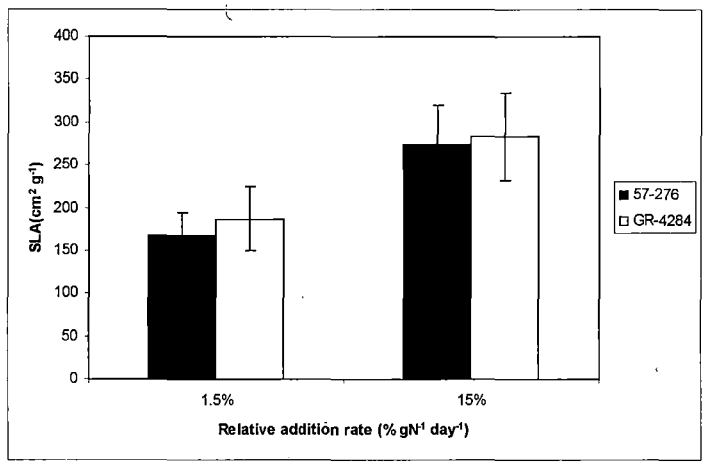


Figure 25. Effects of two N relative addition rate on the specific leaf area ($cm^2\ g^{-1}$) of two hybrid poplar clones, GR-4284 and 57-276

Two approaches were used to determine the effect of SLA on SPAD-leaf N relationship: 1) multiple linear regression using both leaf N concentration and SLA as independent variables and; 2) simple linear regression of leaf N concentration based on leaf area ($mg\ cm^{-2}$).

When the SLA was factored into the SPAD-N relationship, the model became SPAD = A×N + B×SLA + C. The coefficient of determination (R^2) of this model was improved for both clones (Tab. 9). For clone 57-276, R^2 improved from 0.61 to 0.78 while the regression equation for clone GR-4284 increased the R^2 from 0.68 to 0.71. The N and SLA slopes were both significant in the model for clone 57-276. The SLA slope was not significant in the model of GR-4284, however, suggesting that the importance of specific leaf area on SPAD-N relation is clone-dependent.

In the multiple regression model, the slopes of N and SLA were not different between the clones (P_N = 0.31, P_{SLA}= 0.15). This suggested that when SLA was combined into the model, the SPAD-N relation was independent of clone, and thus one model can be used for both clones in the field.

Table 9. The parameters and their p-values in SPAD-N model for two hybrid poplar clones, 57-276 and GR-4284

Clone	N	P	SLA	P	Intercept	R^2
57-276	5.74	<0.0001	-0.07	<0.01	26.32	0.78
GR-4284	7.13	<0.0001	-0.04	0.12	12.52	0.71

The effect of leaf thickness on SPAD readings in the present study was in agreement with those observed in the field study of rice by Peng et al.(1992). Data from a previous field study on poplar

(unpublished data) also supported the observed effect of SLA on SPAD-N correlation. With five N treatments, leaf N concentration ranged from 26 to 34 mg g^{-1}. Clone 57-276 showed higher SPAD readings than GR-4284 when their internal N concentrations were similar and the SLA of GR-4284 was found to be greater than 57-276.

When using SPAD to estimate N level, it is necessary to measure the specific leaf area of thick leaf clones, like 57-276, and combine SLA into the SPAD-N model. The accuracy of SPAD to estimate leaf N concentration will be greatly improved when leaf thickness is considered. However, this may not be necessary for thin leaf clones, such as GR-4284.

When foliar N concentration per cm^2 was plotted against SPAD readings, a better relationship was observed (R^2=0.72) for 57-276 without considering the SLA factor (Tab.10). This is probably because the area based N concentration reduced the effect of leaf thickness in the SPAD-N model. The relationship between SPAD readings and the area-based chlorophyll concentration was also improved. Clone GR-4284 didn't show the improvement observed in 57-276 (Tab.11), but this may be attributed to leaf deformation in this clone caused by a spider mite infestation.

Improvement of correlation between SPAD readings and area-based leaf N concentration was observed in several corn cultivars in the field (Chapman, 1997). The results suggested that area based N concentration was more important than mass based N concentration in determining corn growth. A study on eastern cottonwood clones (Moreau, 2004) didn't support these findings, however, where less precision in predicting area-based N with SPAD-502 meter was found. The disagreement may be because a curvilinear model was used to establish and compare SPAD-N correlations.

Table 10. Linear regression equations among SPAD values, area based N concentration, and area based chlorophyll concentration, for clone 57-276

Variables	Model of 57-276	P value	R^2
SPAD-N	SPAD = 1187.6N + 12.33	<0.01	0.72
SPAD-chl	SPAD = 498.5chl + 5.33	<0.01	0.85
N-chl	N = 0.33chl +0.004	<0.01	0.70

Table 11. Linear regression equations among SPAD values, area based N concentration, and area based chlorophyll concentration for clone GR-4284

Variables	Model of GR-4284	P value	R^2

SPAD-N	SPAD =1283.1N + 8.79	0.02	0.27
SPAD-chl	SPAD = 490.9chl + 14.48	<0.01	0.56
N-chl	N = 0.2chl +0.009	<0.01	0.44

Conclusions of experiment 2

1) The Minolta SPAD-502 chlorophyll meter can estimate the hybrid poplar foliage N concentrations using linear regression equations. However, specific leaf area needs to be factored into the SPAD-N model, especially for thick leaf clones, like 57-276.

2) At similar leaf N concentration, SPAD readings increase with leaf thickness, as a result of measurement principles of the SPAD-502 meter.

3) Using area-based leaf N and chlorophyll concentration to establish the SPAD-N relationship can improve the accuracy of the regression model.

4.3 Conclusion

From the present study, it is concluded that growth of hybrid poplars in response to N supply differed by clone. Precise N management tailored to the clones is justified to maximize growth while minimizing N fertilization. Clone GR-4284 is more adaptable to severe nitrogen deficit, and therefore expected to grow better than 57-276. On the other hand, N fertilization can cause greater growth increase in 57-276. To develop fertilization guidelines, further research on root development, N uptake ability and respiration of the clones is needed. Additional clones as well as intermediate N levels should be studied.

The SPAD-502 chlorophyll meter, with leaf morphology information, adequately estimated hybrid poplar leaf N level. A linear relationship between SPAD reading and foliar N level can be established in both field and greenhouse. Field testing of a wide range of clones is needed to further validate the present findings.

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APPENDIX

Appendix 1□ List of parameters used with their symbols, units and definitions

Symbol	Parameter	Unit	Calculation (when applicable)
DW	Dry weight	g	-
GRC	Growth response coefficient	dimensionless	$\frac{\ln LWR_h - \ln LWR_l}{\ln RGR_h - \ln RGR_l}$
LA	Leaf area	cm^{-2}	-
LAR	Leaf area ratio	$cm^2\ g^{-1}$	LA/plant DW
LNC	Leaf N concentration	mg g^{-1}or mg cm^{-2}	-
LNR	Leaf N ratio	dimensionless	Leaf N content/plant N content
LWR	Leaf weight ratio	dimensionless	Leaf DW/plant DW
NAR	Net assimilation rate	g cm^{-2} day^{-1}	RGR/LAR
NUE_{PH}	Photosynthetic N use efficiency	µmol CO_2 gN^{-1} s^{-1}	A/LNC
RAR	Relative addition rate	g g^{-1}N day^{-1}	-
RGR	Relative growth rate	g g^{-1} day^{-1}	1/DW × dDW / dT
RUR	Relative uptake rate	g N g^{-1} N day-1	1/Ncontent× dNcontent / dT
Pn	N productivity	g g^{-1} N day^{-1}	RGR/PNC
Pnl	Leaf N productivity	g g^{-1} leafN day^{-1}	NAR/LNC
PNC	Plant N concentration	mg g^{-1}or mg cm^{-2}	-
A	Photosynthesis rate	µmol CO_2 m^{-2} s^{-1}	-
SLA	Specific leaf area	$cm^2\ g^{-1}$	LA/leaf DW
T	time	days	-

Appendix2 □ The P-value of parameter changes from 1.5% to 10% RAR

Parameters	57-276	GR-4284	N-clone Interaction
RGR	<0.0001	<0.0001	0.14
SLA	<0.0001	<0.0001	0.15
LWR	<0.0001	<0.0001	0.05
NAR	0.0009	0.17	0.09
A	<0.0001	<0.0001	0.69
Pn	0.008	0.22	0.07
LNR	<0.0001	<0.0001	0.06
Pnl	0.0008	0.22	0.13
NUE_{PH}	0.0035	0.0022	0.56
PNC	<0.0001	<0.0001	<0.01